

Table 1

Immunization of rabbits and rhesus monkeys with anti-id BR55-2 #E4 or
unspecific mouse IgG1

Relative titer increase at week 9 compared to 1:4 diluted preserum
(Cell ELISA)

	Rabbit		Monkey	
	anti-id BR55-2	mouse IgG1	anti-id BR55-2	mouse IgG1
SKBR5 (Y+)	1000	16	1000	8
WM9 (Y-)	3	n.t.	25	n.t.

Table 2

Boost immunization of rhesus monkeys with anti-id BR55-2 #E4 or
unspecific mouse IgG1

Blinding of monkey serum Ig to a SKBR5-membrane preparation (ELISA)
Relative titer increase compared to 1:4 diluted preserum

	9 weeks after first course	2 years after first course	1 week after first boost	4 weeks after first boost
anti-id BR55-2	40	1	80	50
mIgG1	1	2	n.t.	2

Table 3

Binding of rhesus monkey's serum Ig to HIV-infected/non-infected PALL-cell line
(Indirect immunofluorescence, IFA-Anti-HIV1-Kit Waldheim)

Immunization of rhesus monkeys with anti-id BR55-2 #E4 unspecific mouse-IgG1				Human serum controls (Kit)	
PALL:	HIV+	HIV-	HIV+	HIV-	
<u>preserum</u>					
conc.	1	1	1	1	
1:10	1	1	1	1	
<u>serum 9 weeks after first immunization</u>					
conc.	3	1	1-2	1	
1:10	3	1	1-2	1	
<u>PALL: HIV+ HIV-</u>					
<u>HIV-neg. human serum</u>					
	0	0			
<u>HIV-pos. human serum</u>					
	5	0			

0-5 = intensity of fluorescence (anti-human IgG-FITC)

0 = no fluorescence

5 = maximum fluorescence

Table 4

Binding of rhesus monkey Ig immunopurified on anti-id BR55-2 #E4 (AB3) and BR55-2 / murine IgG3 (AB1) to HIV-infected/non-infected PALL-cell line (indirect immunofluorescence, IFA-Anti-HIV1-Kit Waldheim)

AB3 (20 µg/ml)		BR55-2 / murine IgG3 (20 µg/ml)	
PALL: HIV+	HIV-	HIV+	HIV-
3	0	2	0

Human serum controls (Kit)	
PALL: HIV+	HIV-
HIV-neg. human serum	
0	0

HIV-pos. human serum	
5	1

0-5 = intensity of fluorescence (anti-human IgG-FITC)
 0 = no fluorescence
 5 = maximum fluorescence

Figure 1
SDS - PAGE

St

E4

C11

B3

B9

G6

G9

St

E4

C11

B3

B9

G6

G9

St

E4

C11

B3

B9

G6

G9

St

E4

C11

B3

B9

G6

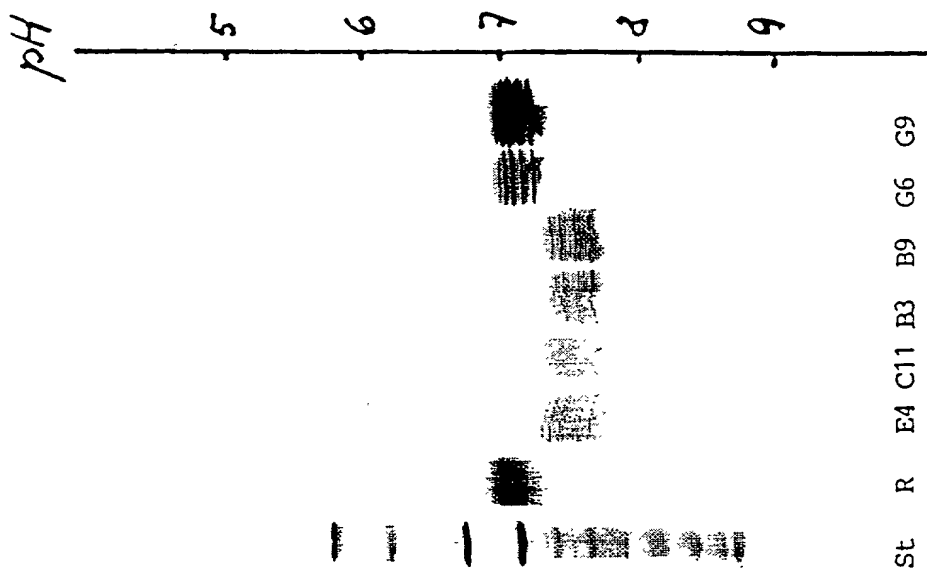
G9

St = Standards

E4, C11, B3, B9, G6, G9 =
anti-id BMS5-2 Nabs

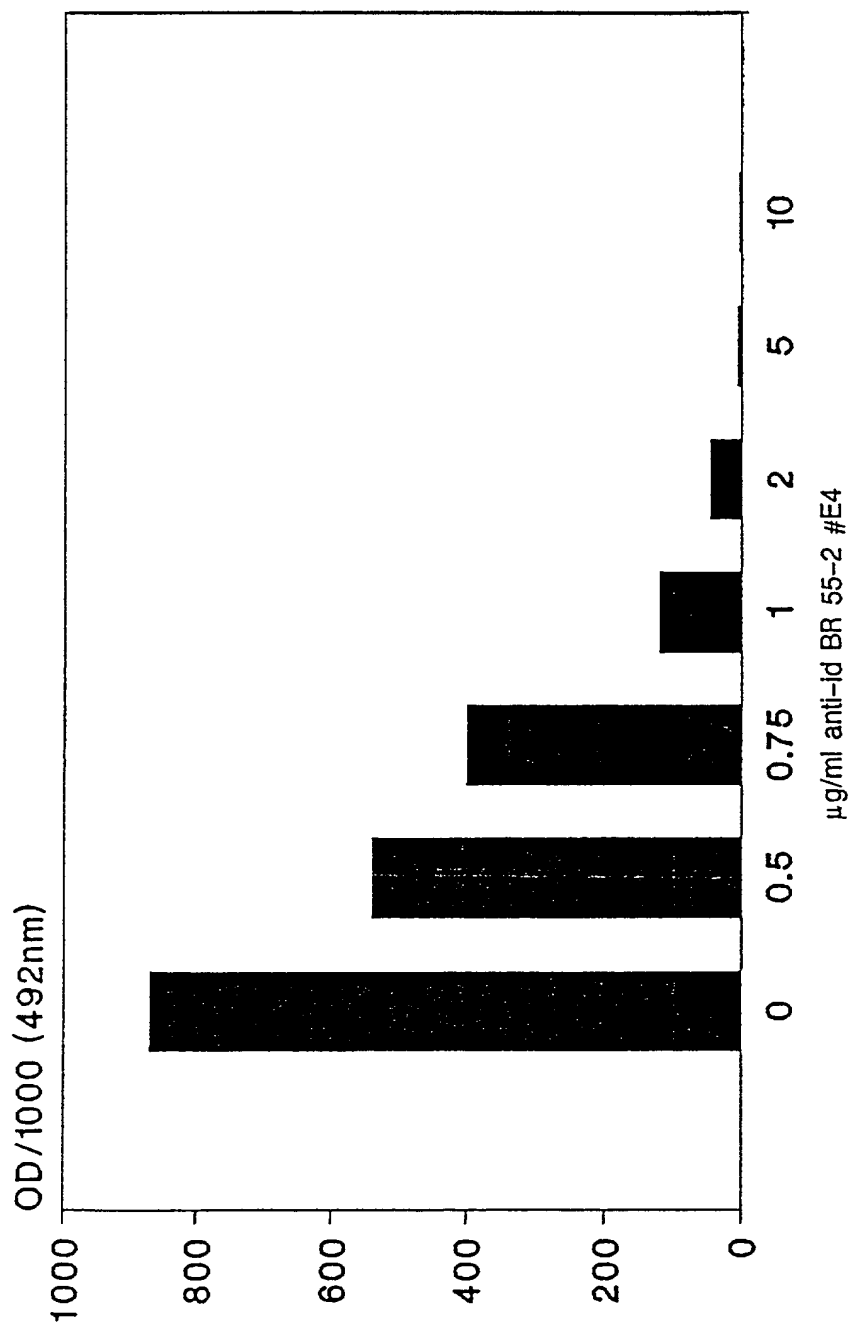
St E4 C11 B3 B9 G6 G9 St E4 C11 B3 B9 G6 G9
reducing conditions non-reducing conditions

Figure 2
Isoelectric Focussing



St = Standard.
R = Reference Mab
E4, C11, B3, B9, G6 and G9 = anti-id BR55-2 Mabs

Figure 3
Binding of BR55-2/murine IgG3 to SKBR5 cell line
Inhibition by anti-id BR55-2 #E4



BR 55-2/murine IgG3: 1 µg/ml

Figure 4
Binding of rabbit Ig to SKBR5 before and after immunization with
anti-Id BR55-2 #E4 (Cell-ELISA)

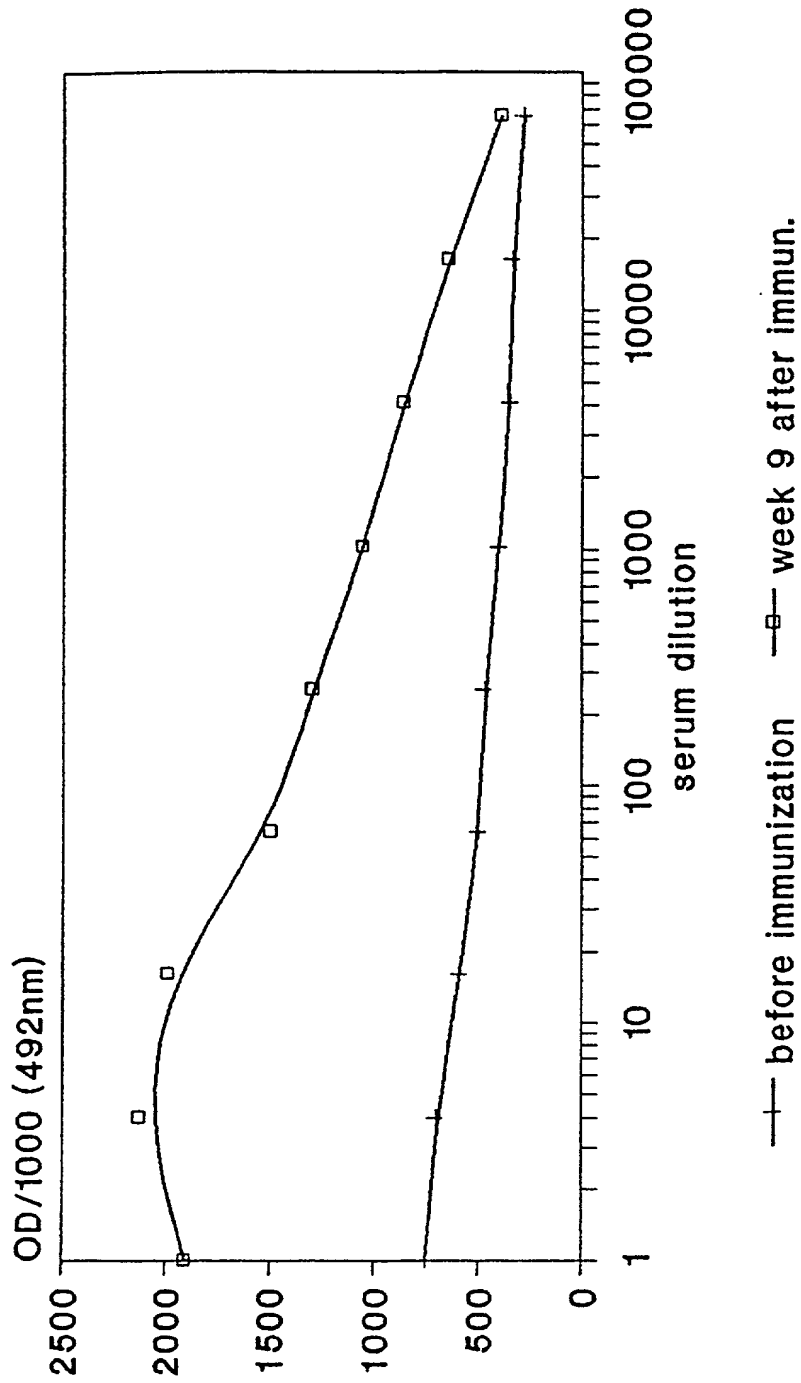


Figure 5
Binding of rhesus monkey Ig to SKBR5 before and after immunization with
anti-id BR55-2 #E4 (Cell-ELISA)

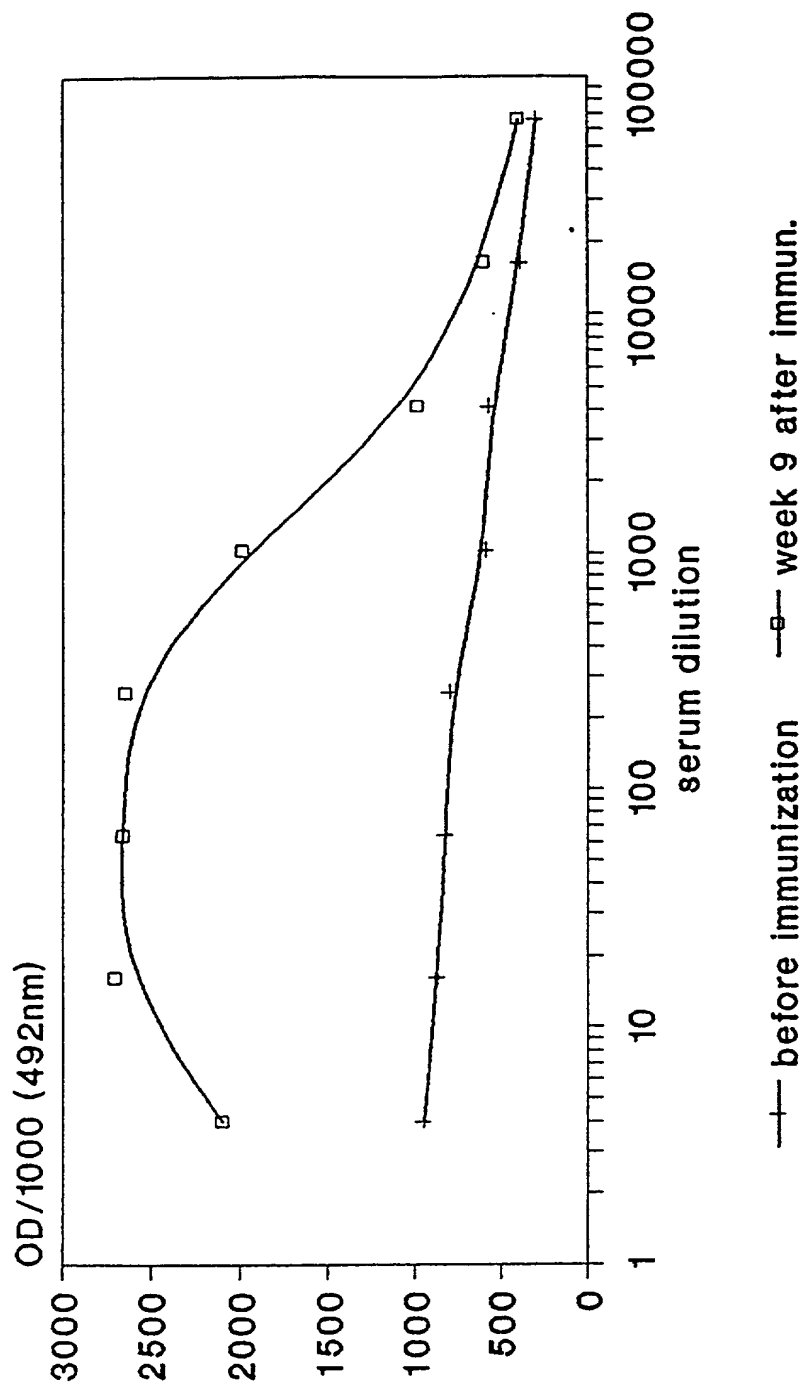


Figure 6
Immunization of monkey 246 with anti-id BR55-2#E4
Serum Ig binding to anti-id BR55-2#E4
(ELISA)

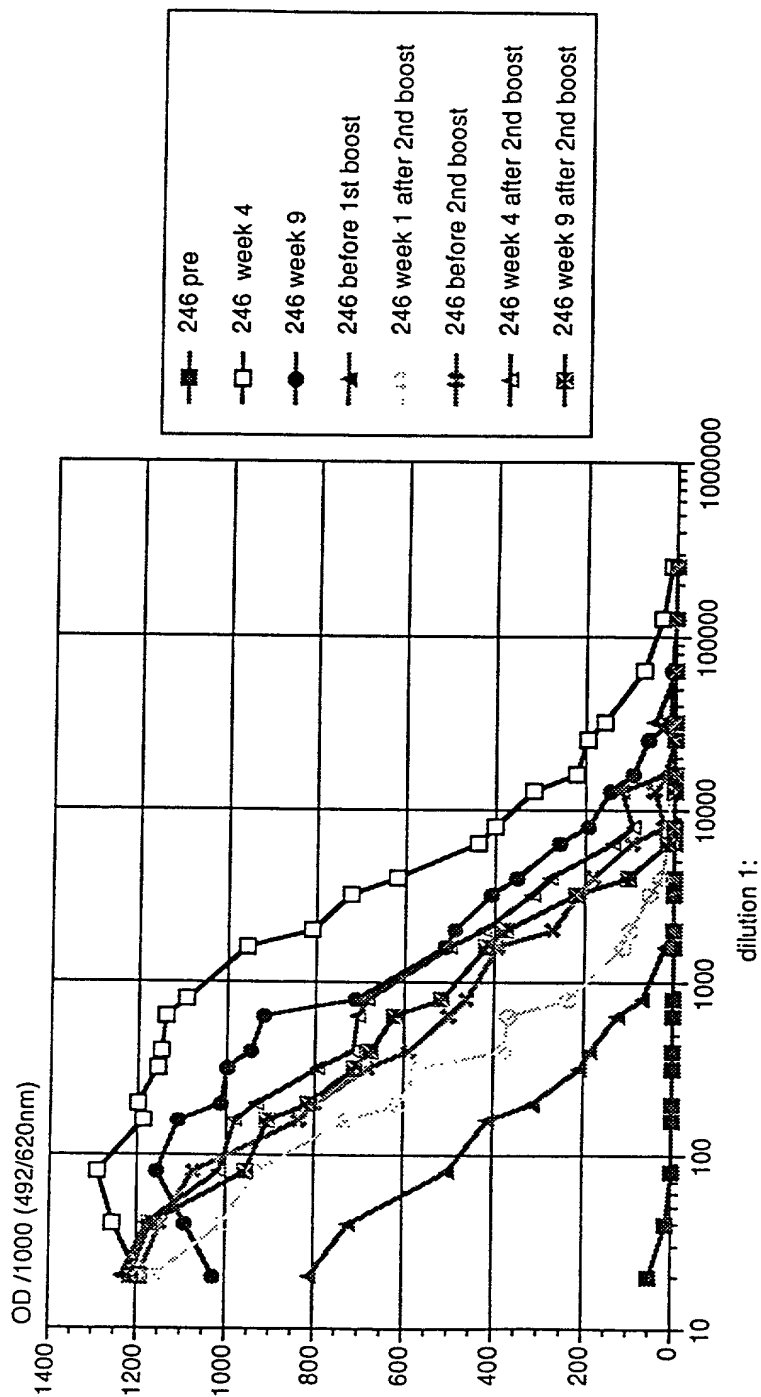


Figure 7
Immunization of monkey 287 with anti-id BR55-2#E4
Serum Ig binding to anti-id BR55-2#E4
(ELISA)

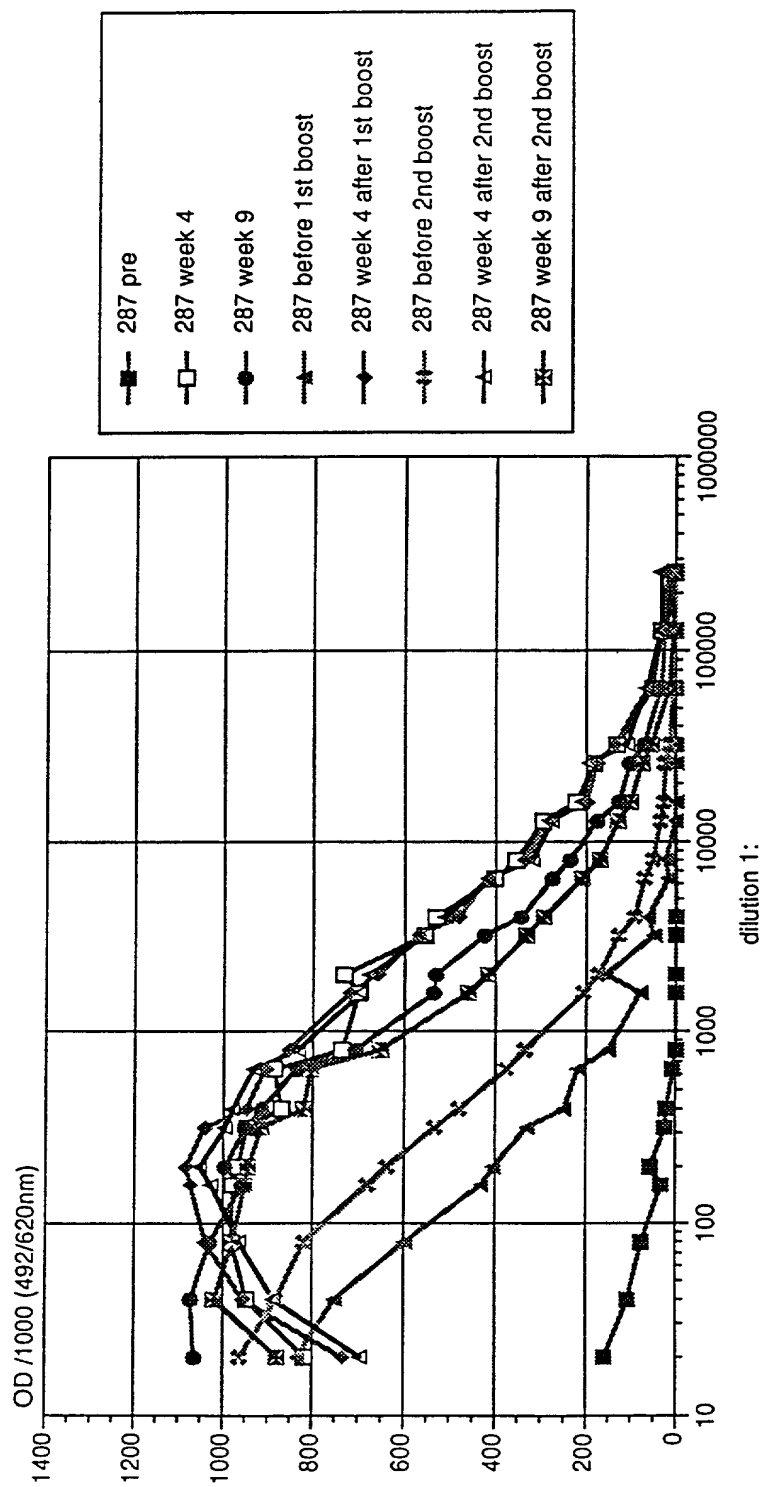


Figure 8
 Immunization of monkey E23 with anti-id BR55-2#E4
 Serum Ig binding to anti-id BR55-2#E4
 (ELISA)

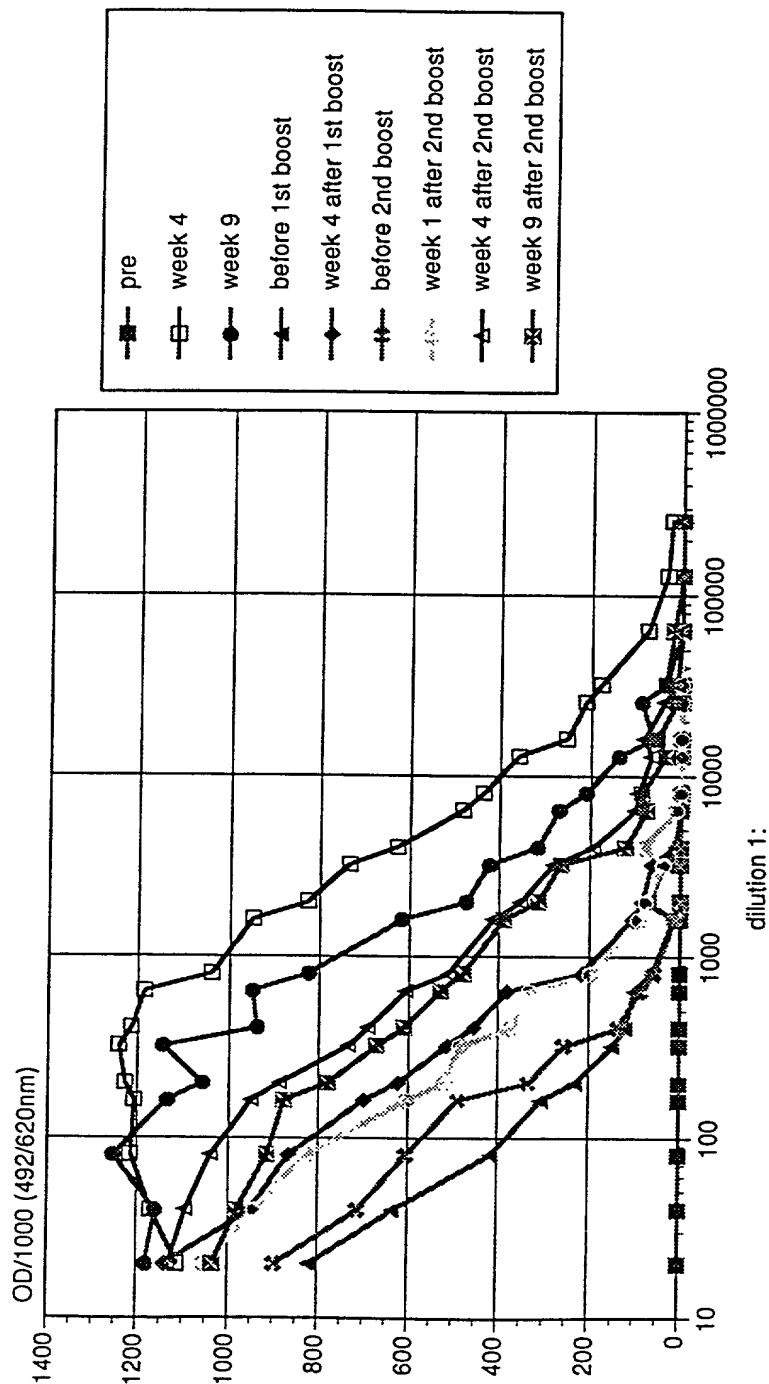


Figure 9
Immunization of monkey 215 with irrelevant mouse IgG1
Serum Ig binding to anti-id BR55-2#E4 (ELISA)

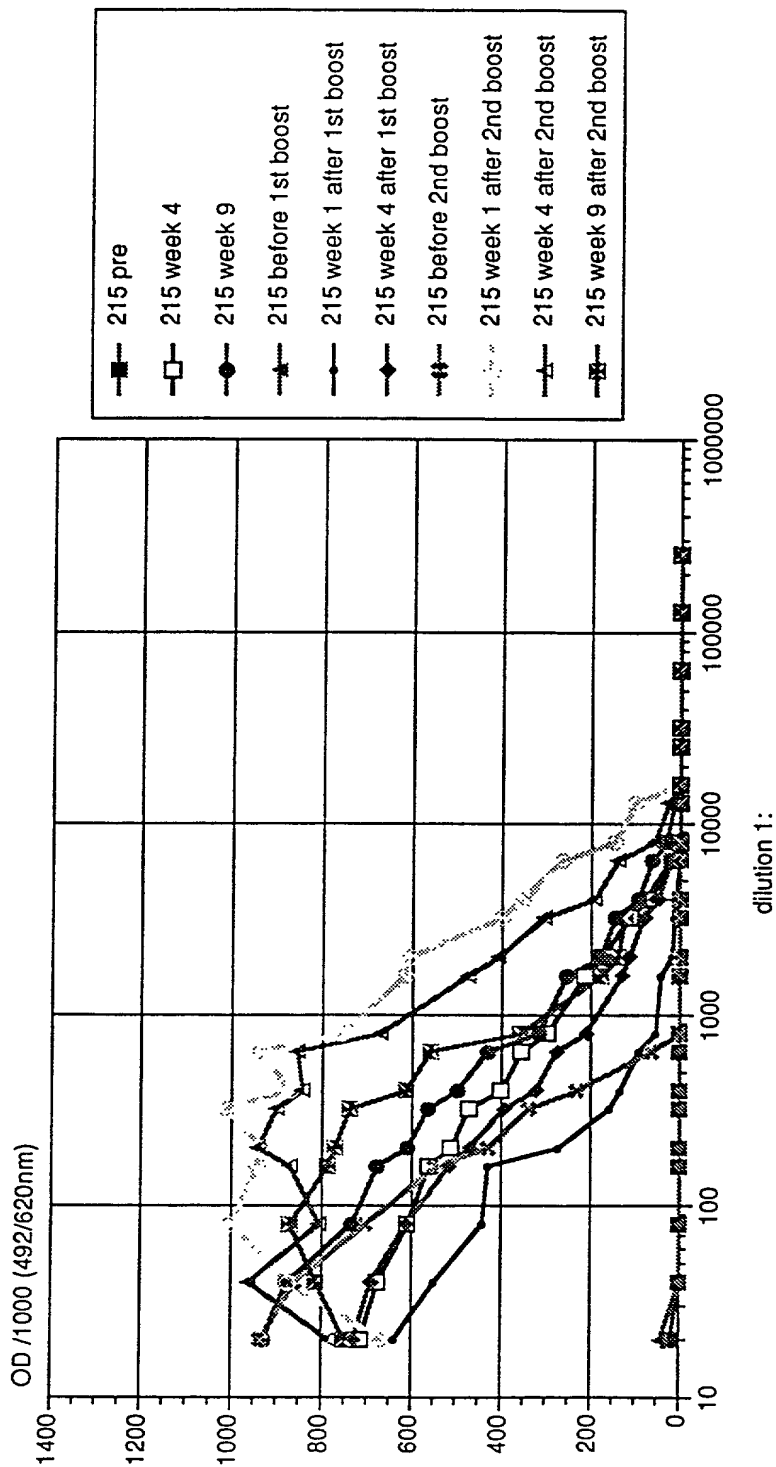


Figure 10
Immunization of monkey 252 with irrelevant mouse IgG1
Serum Ig binding to anti-id BR55-2#E4 (ELISA)

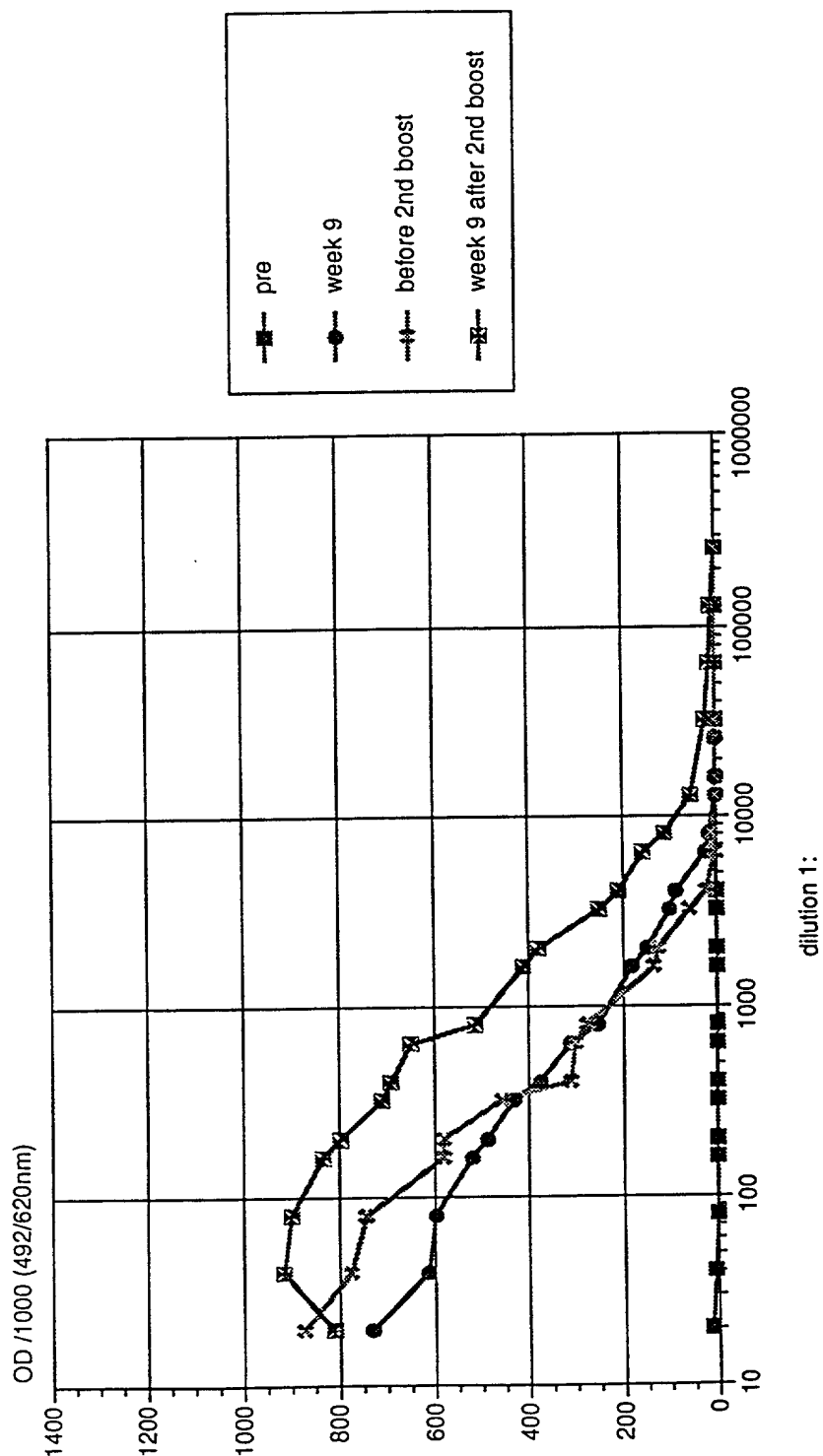


Figure 11
 Immunization of monkey 246 with anti-id BR55-2#E4
 Serum Ig binding to SKBR5 breast cancer cells
 (cell- ELISA)

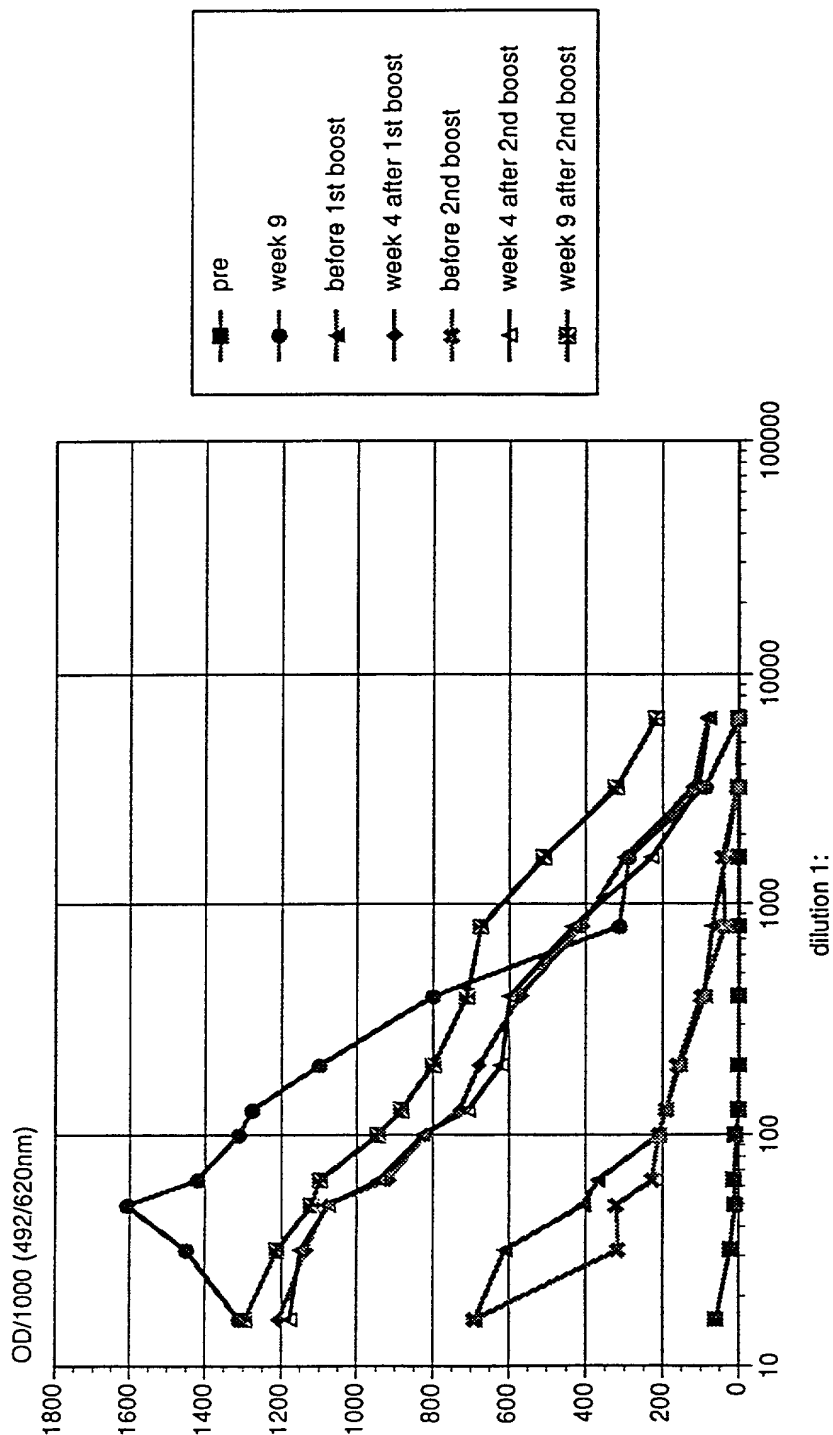
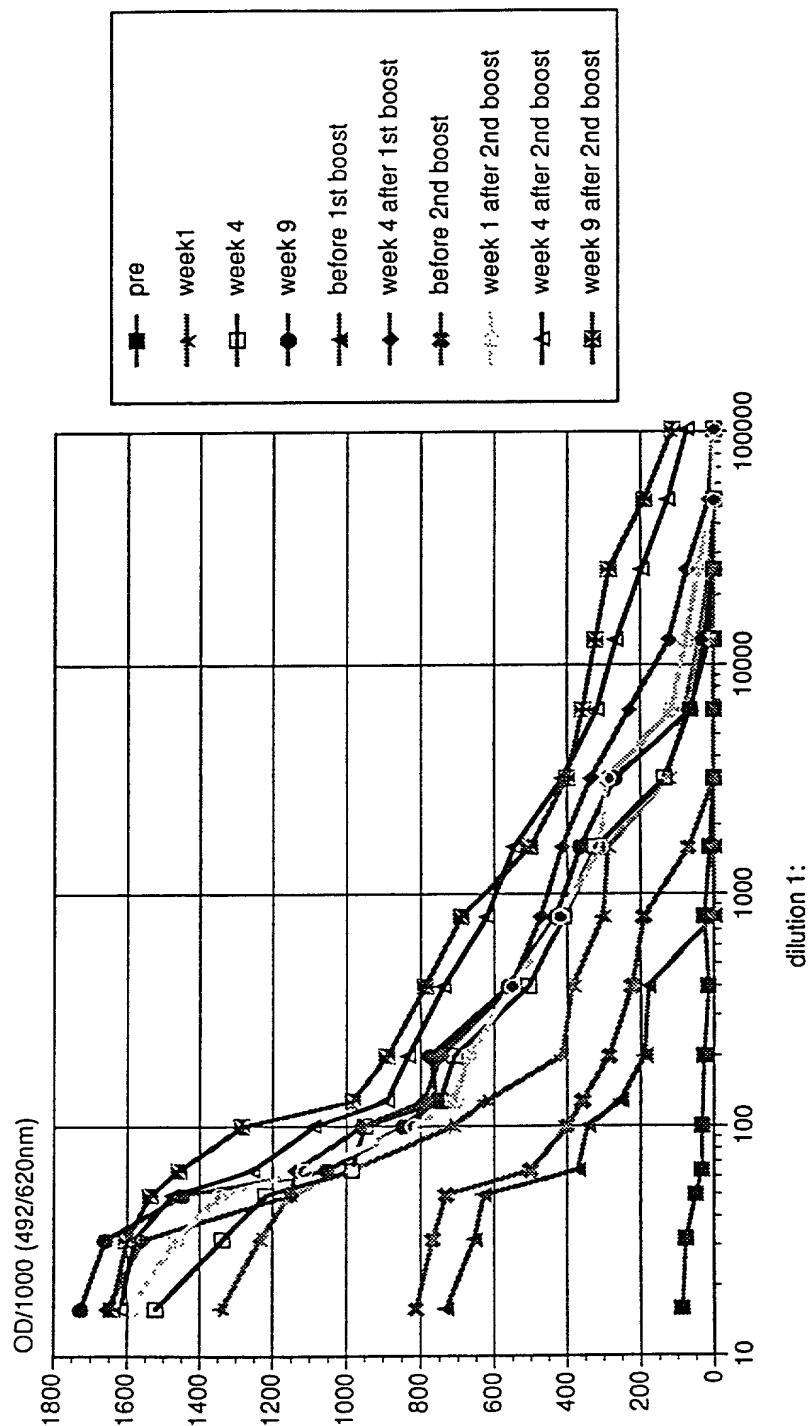


Figure 12
Immunization of monkey 287 with anti-id BR55-2#E4
Serum Ig binding to SKBR5 breast cancer cells
(cell-ELISA)



Immunization of monkey E23 with anti-id BR55-2#E4
Serum Ig binding to SKBR5 breast cancer cells
(cell- ELISA)

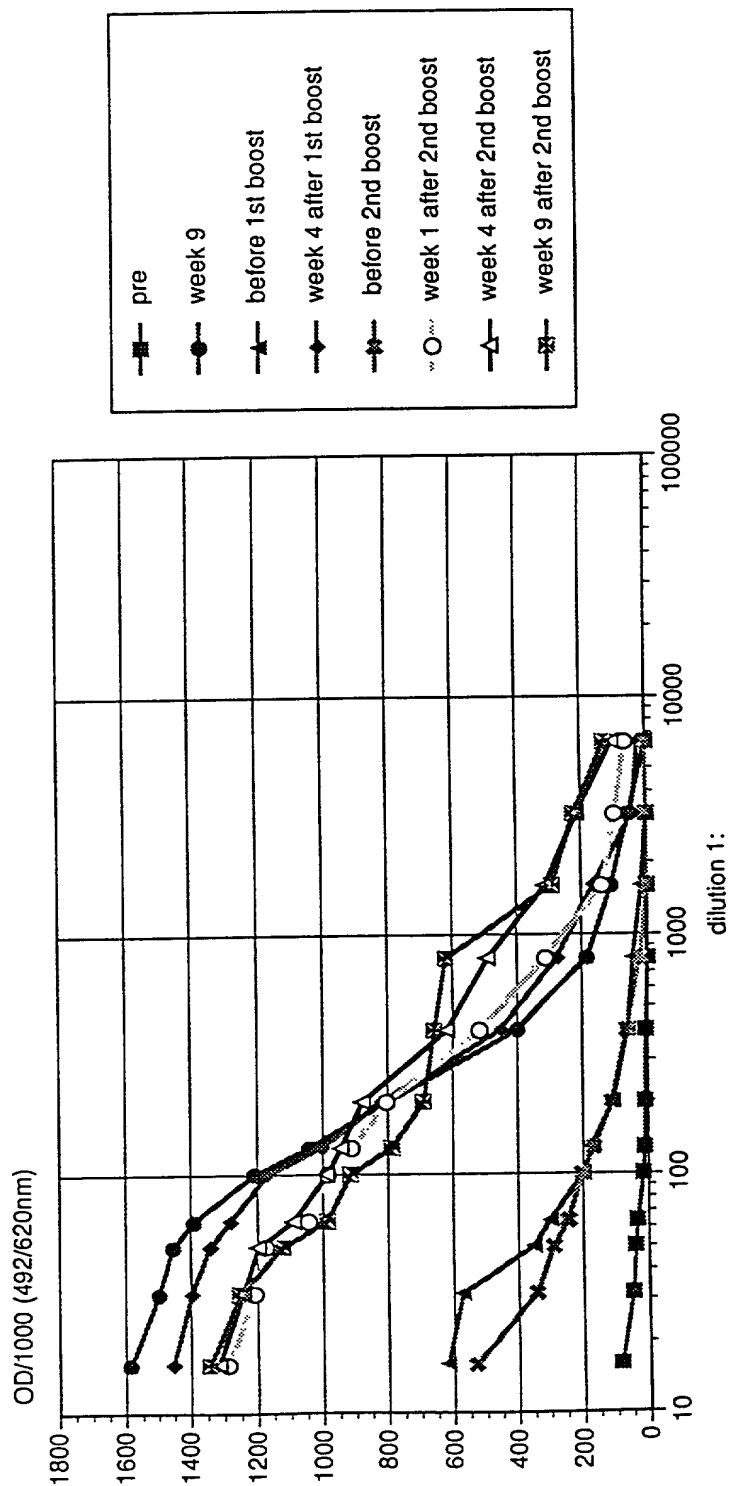


Figure 14
 Immunization of monkey 215 with irrelevant mouse IgG1
 Serum Ig binding to SKBR5 breast cancer cells
 (cell- ELISA)

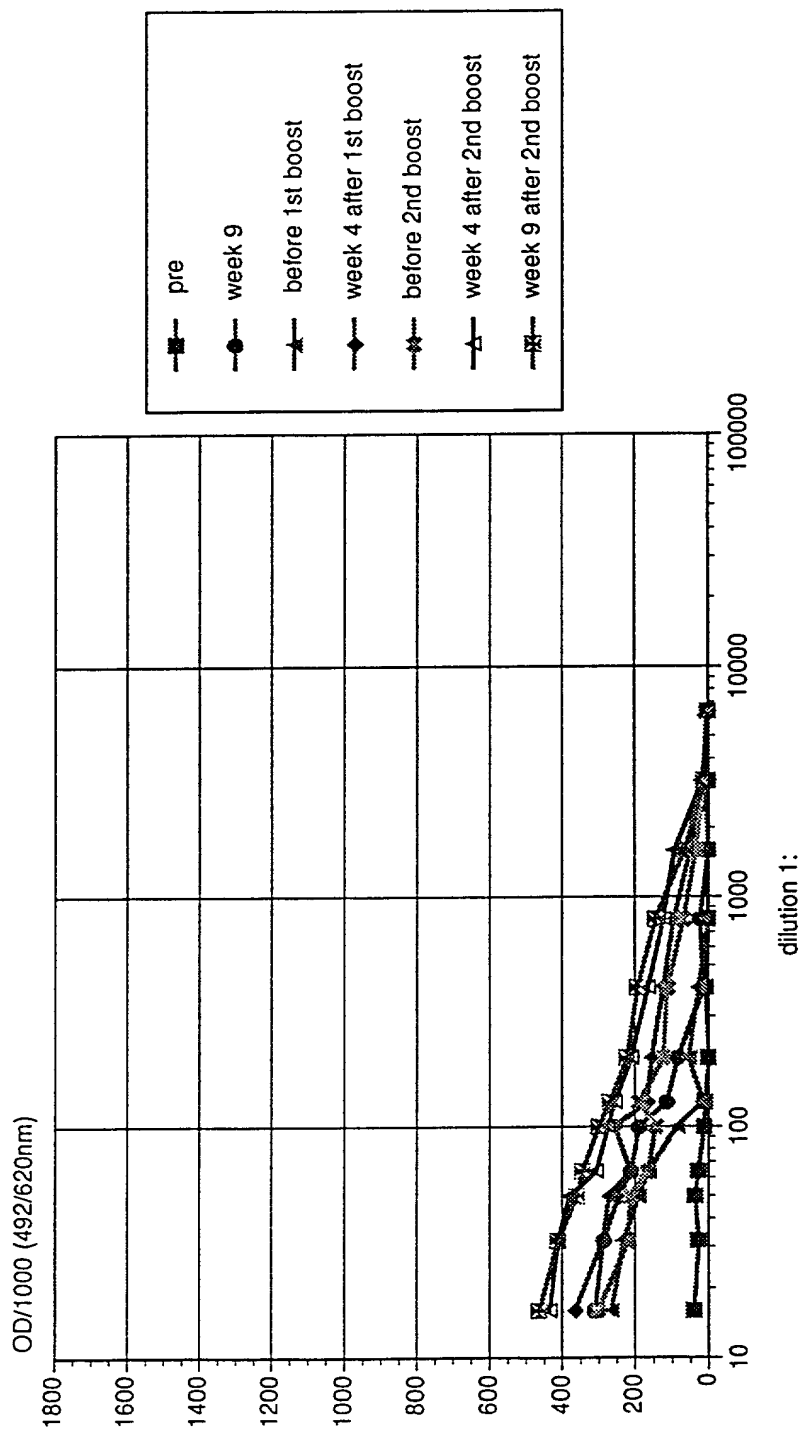


Figure 15

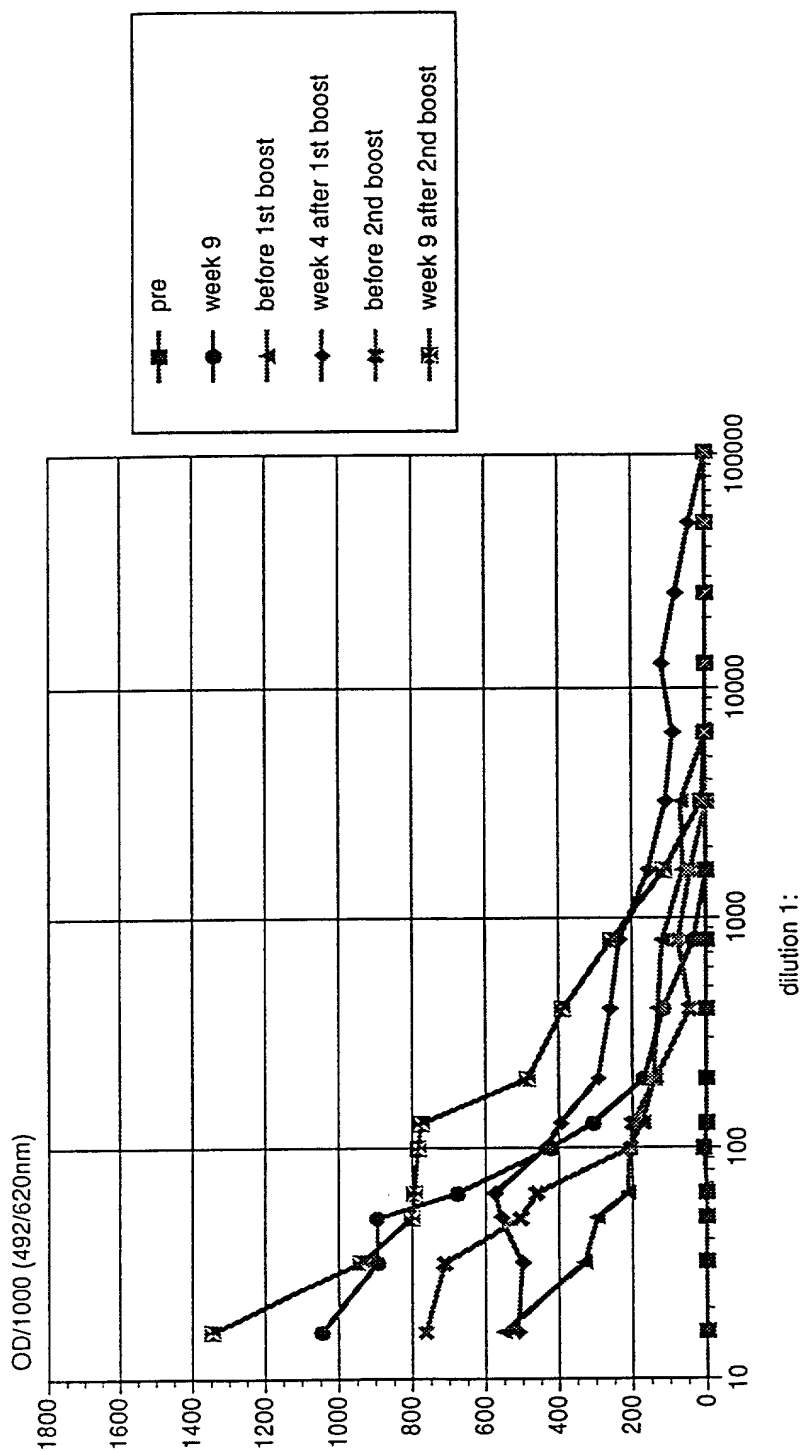


Figure 17
Immunization of monkey E 23 with anti-id BR55-2#E4
Serum Ig binding to SW2 small cell lung cancer cells
(cell- ELISA)

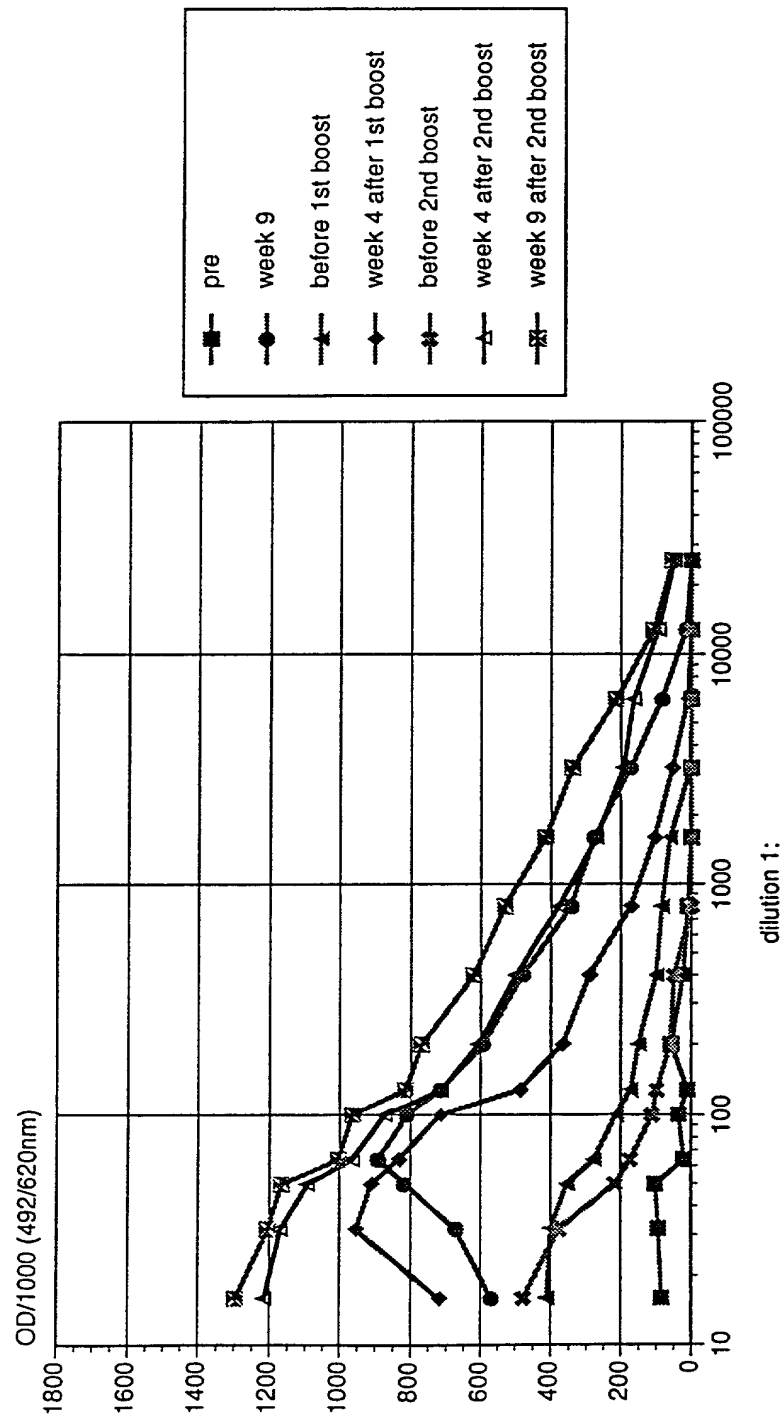


Figure 18

Immunization of monkey 215 with irrelevant mouse IgG1
Serum Ig binding to SW2 small cell lung cancer cells
(cell- ELISA)

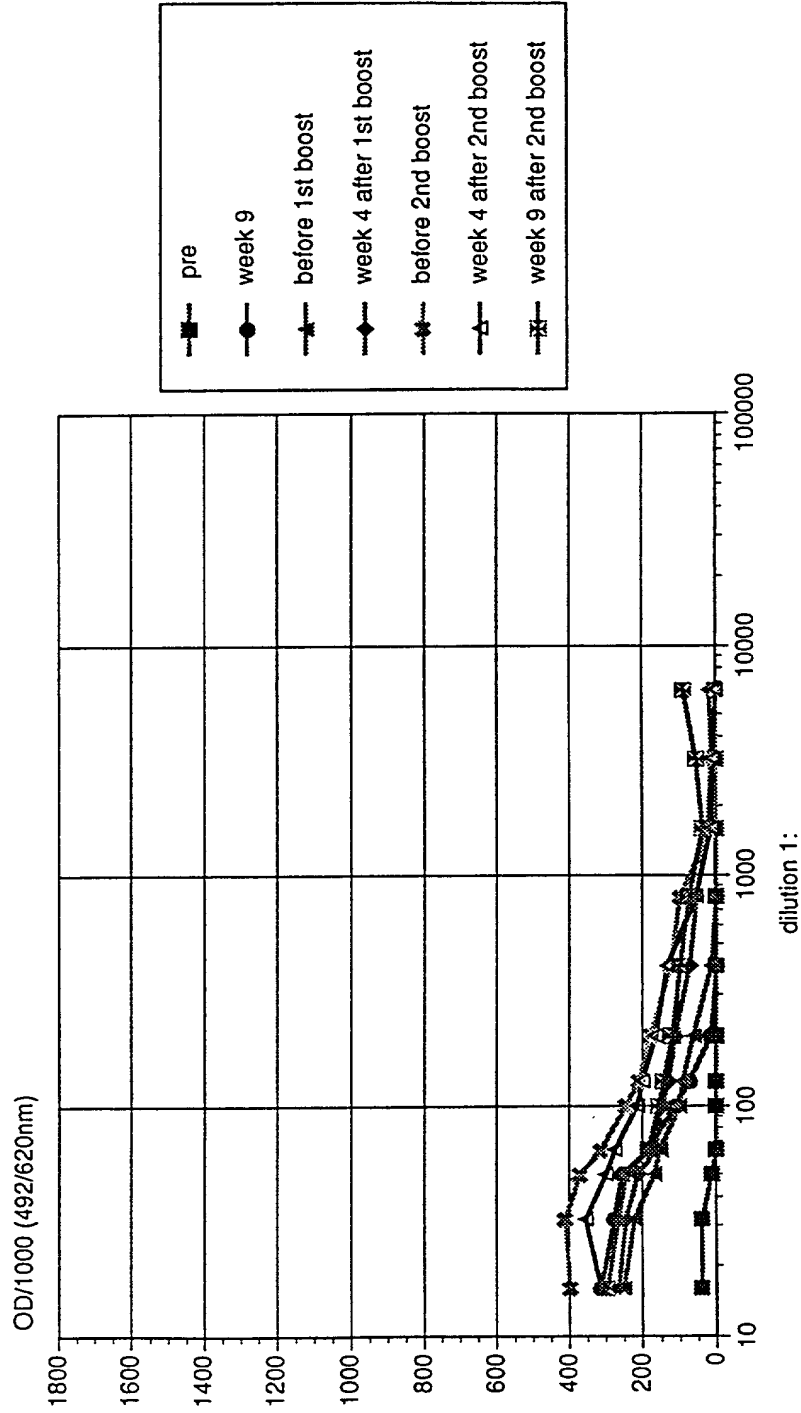


Figure 19
 Immunization of monkey E 23 with anti-id BR55-2#E4
 Serum Ig binding to SW 948 colon cancer cells
 (cell- ELISA)

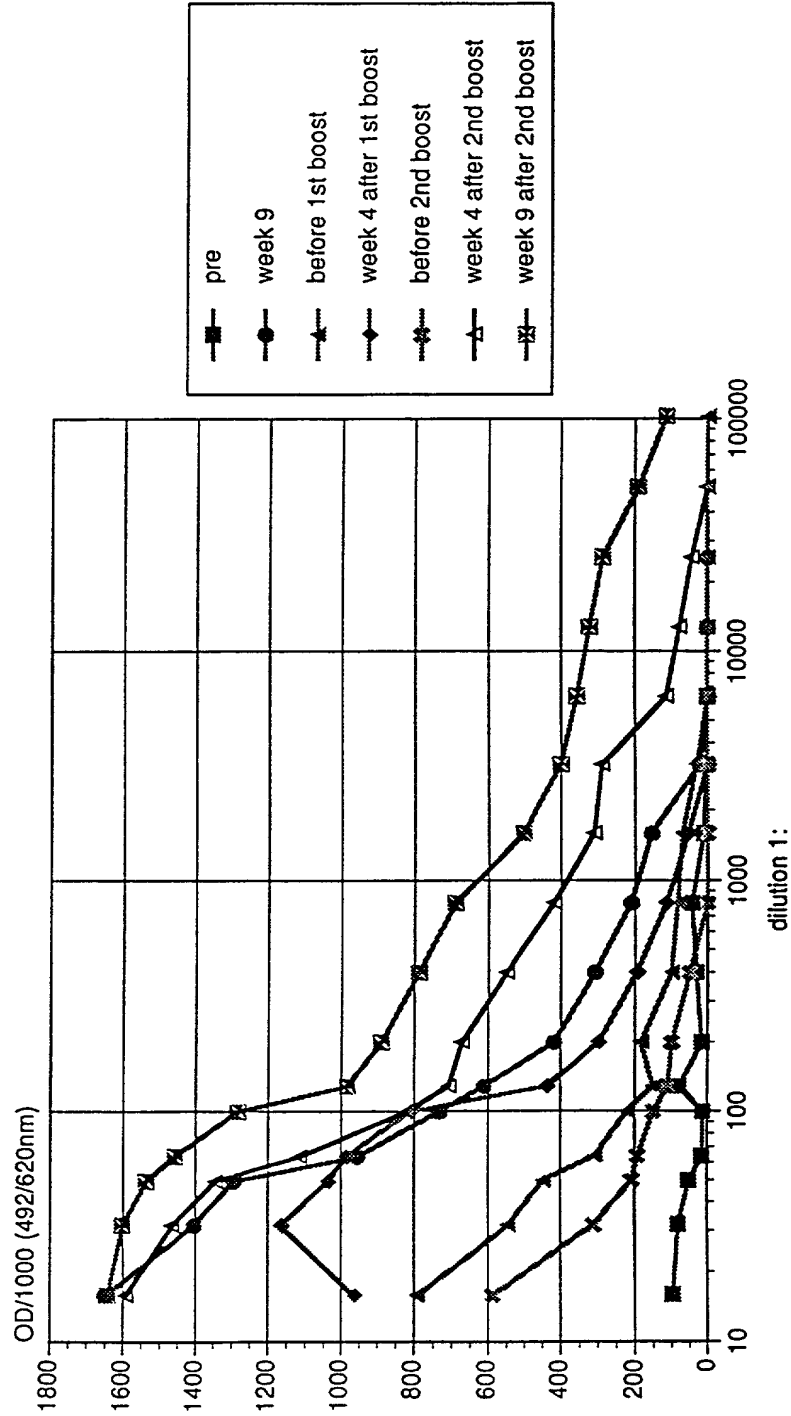


Figure 20
Immunization of monkey E23 with anti-id BR55-2#E4
Serum Ig binding to WM 9 melanoma cells
(cell- ELISA)

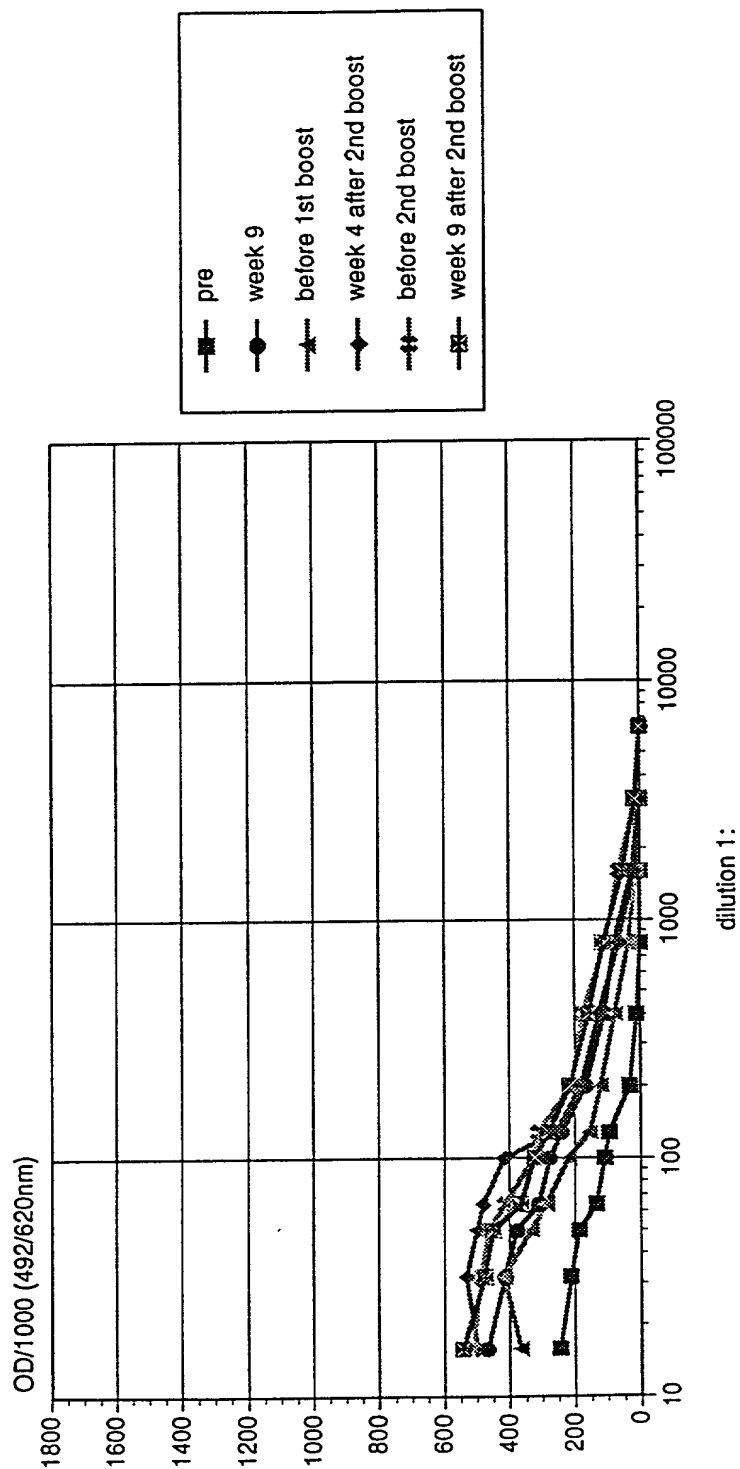


Figure 21
Rhesus monkey Ig immunopurified on anti-id BR55-2#E4*:
binding to anti-id BR55-2#E4
(ELISA)

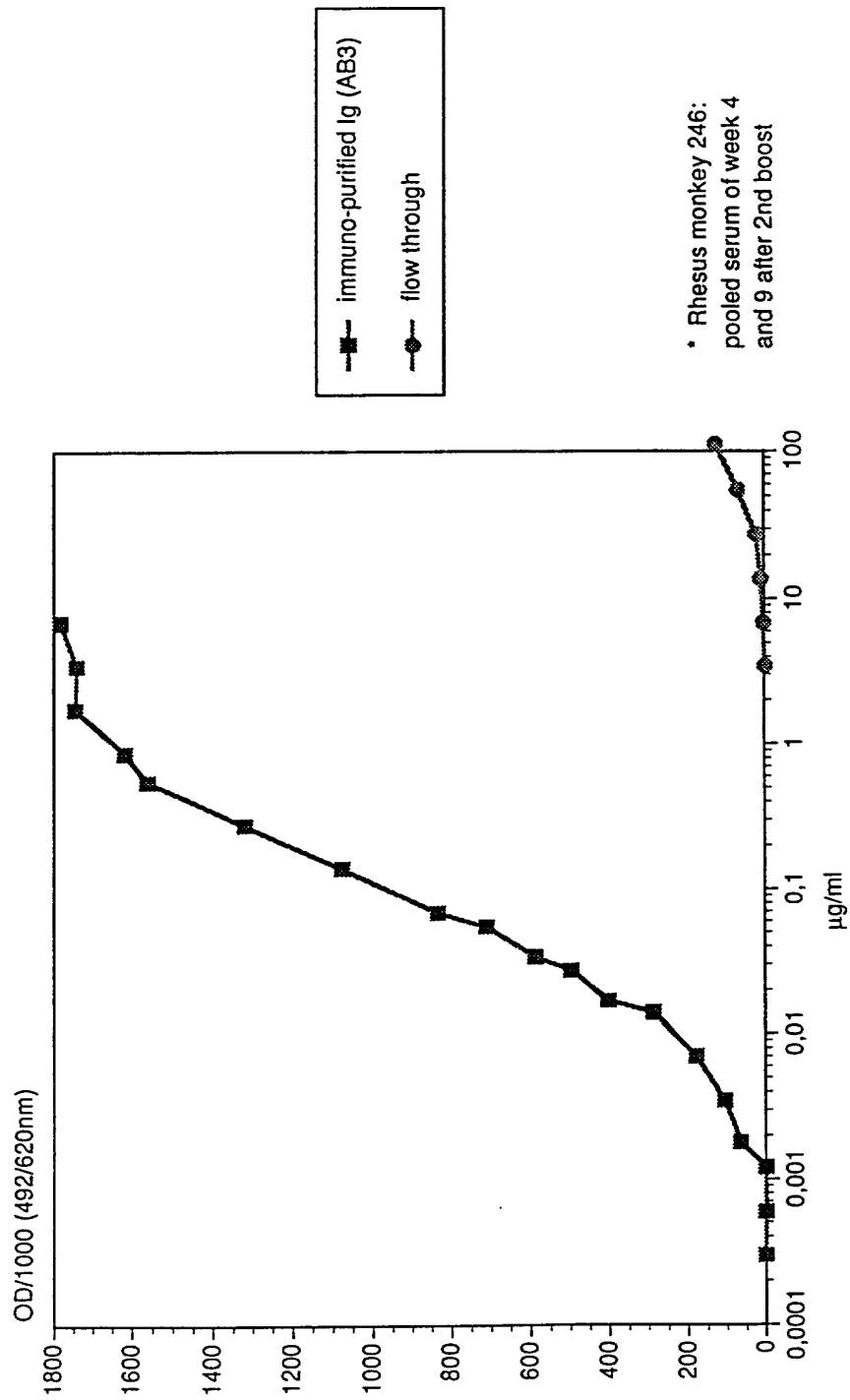


Figure 22

Rhesus monkey Ig immunopurified on
anti-id BR55-2#E4*:
binding to SKBR5 breast cancer cells (cell-ELISA)

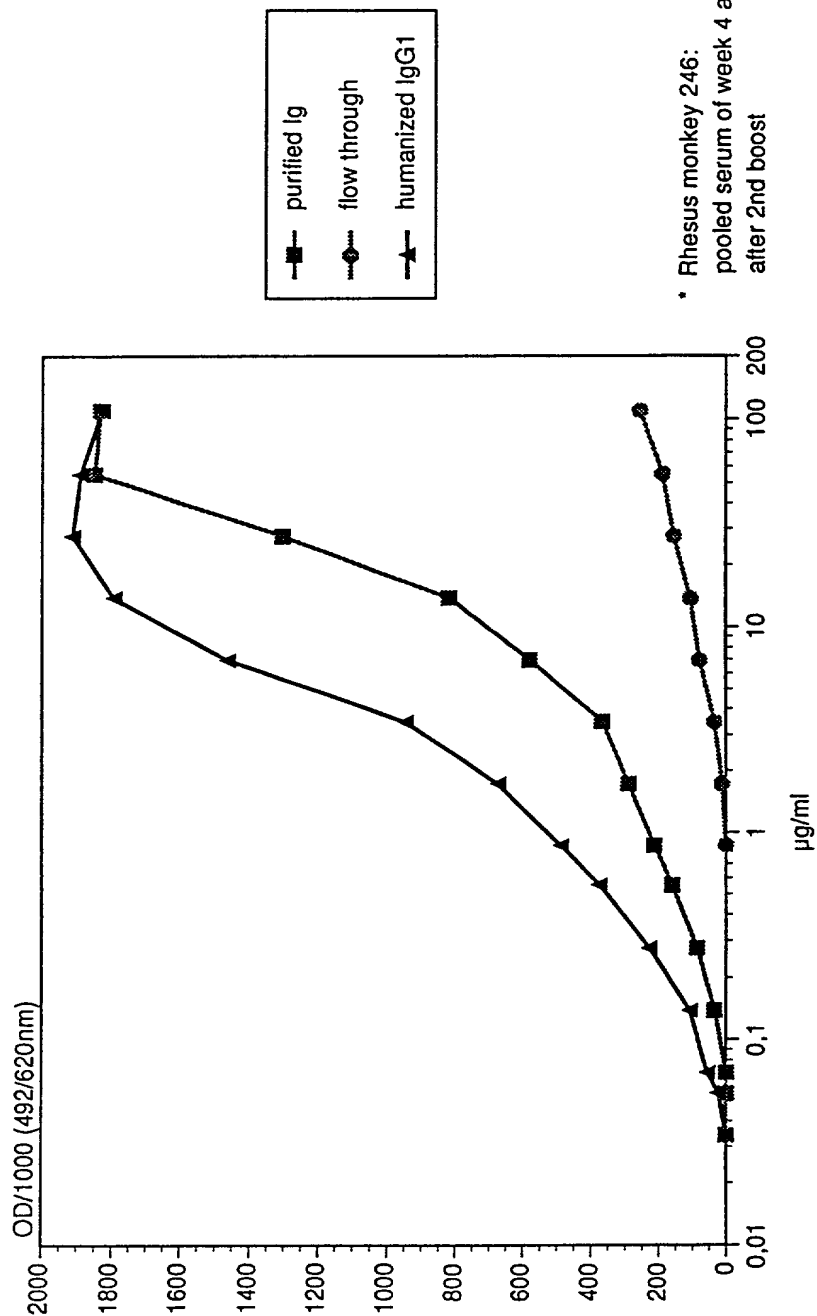


Figure 23
Rhesus monkey Ig immunopurified on anti-id BR55-2#E4*:
binding to WM9 (melanoma) and SKBR5 (breast cancer)
cells (cell-ELISA)

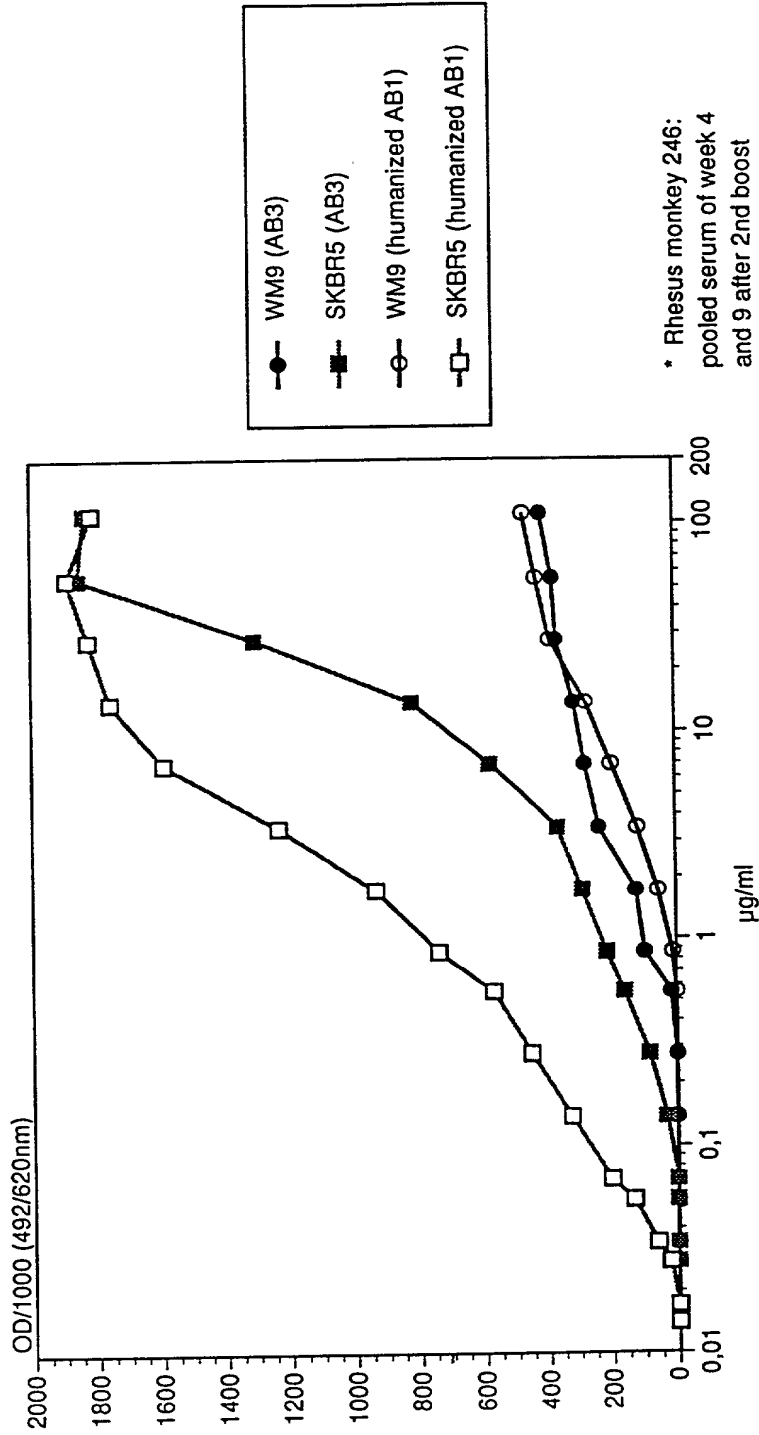


Figure 24
Rhesus monkey Ig immunopurified on
anti-id BR55-2#E4*:
binding to SKBR5 - and WM9 - cell membranes
(ELISA)

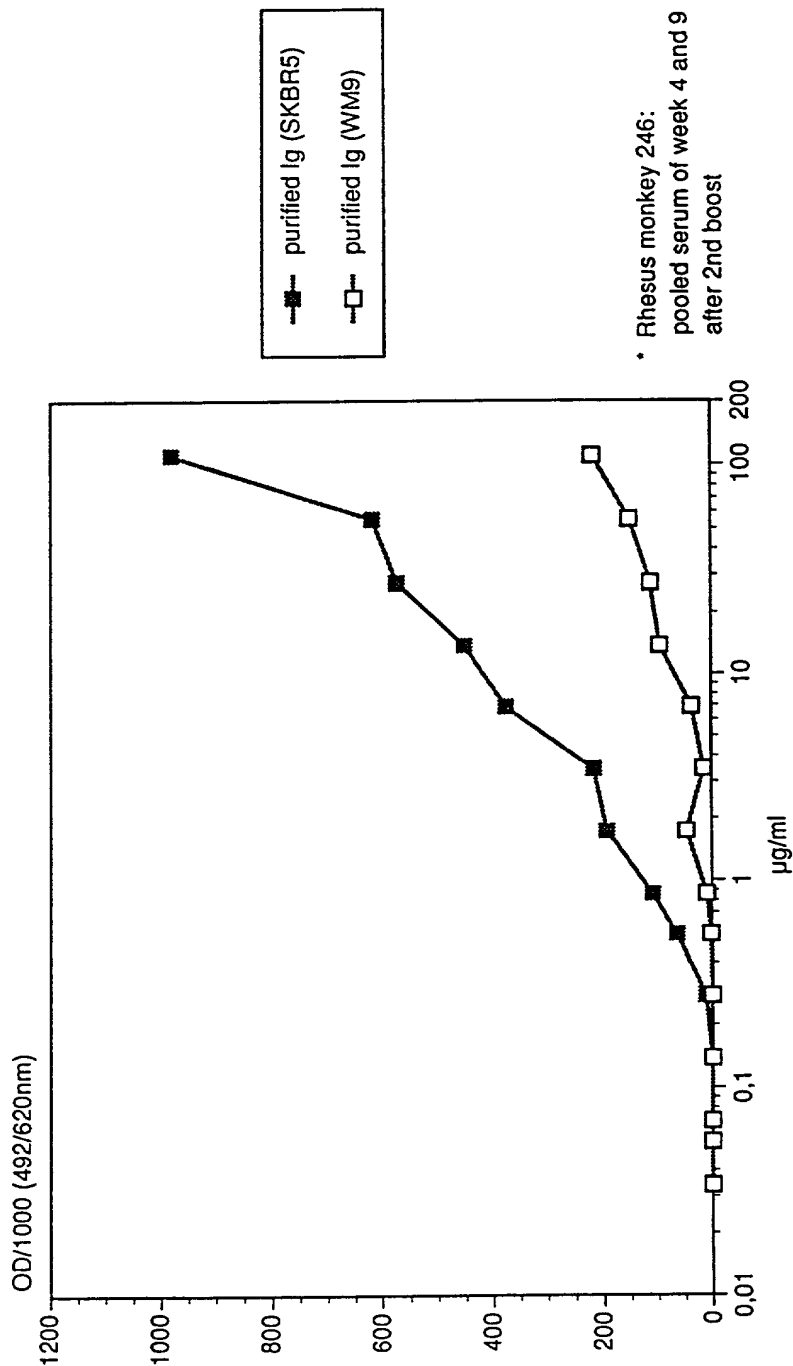


Figure 25
Rhesus monkey Ig immunopurified on
anti-id BR55-2#E4*:
binding to different cell lines (cell-ELISA)

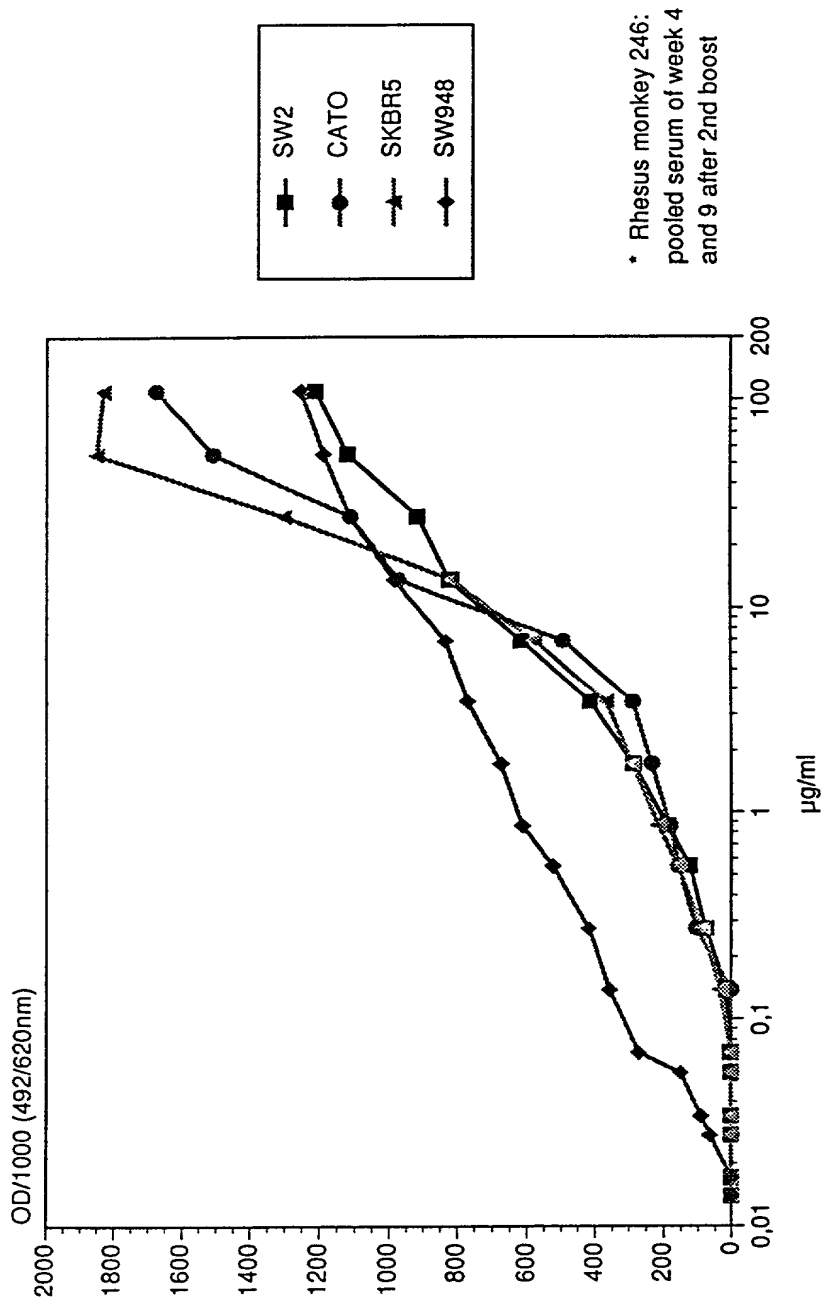


Figure 26
ADCC to SKBR5 breast cancer cell line mediated by
human PBMC and BR55-2/murine IgG3 or immunopurified
Ig of a rhesus monkey*
immunized with anti-id BR55-2#E4

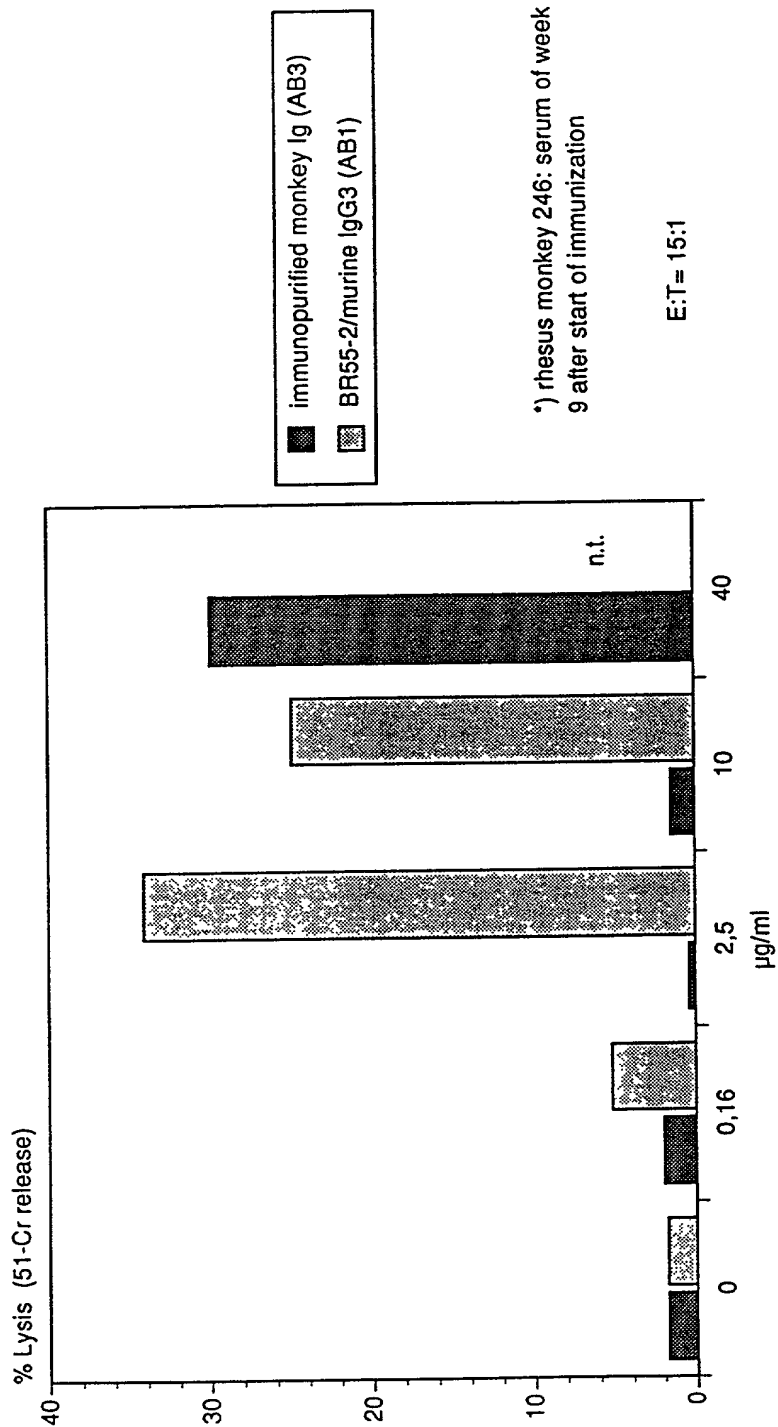


Figure 27
ELISA for the determination of
BR 55-2/murine IgG3 in human serum
Standard curve

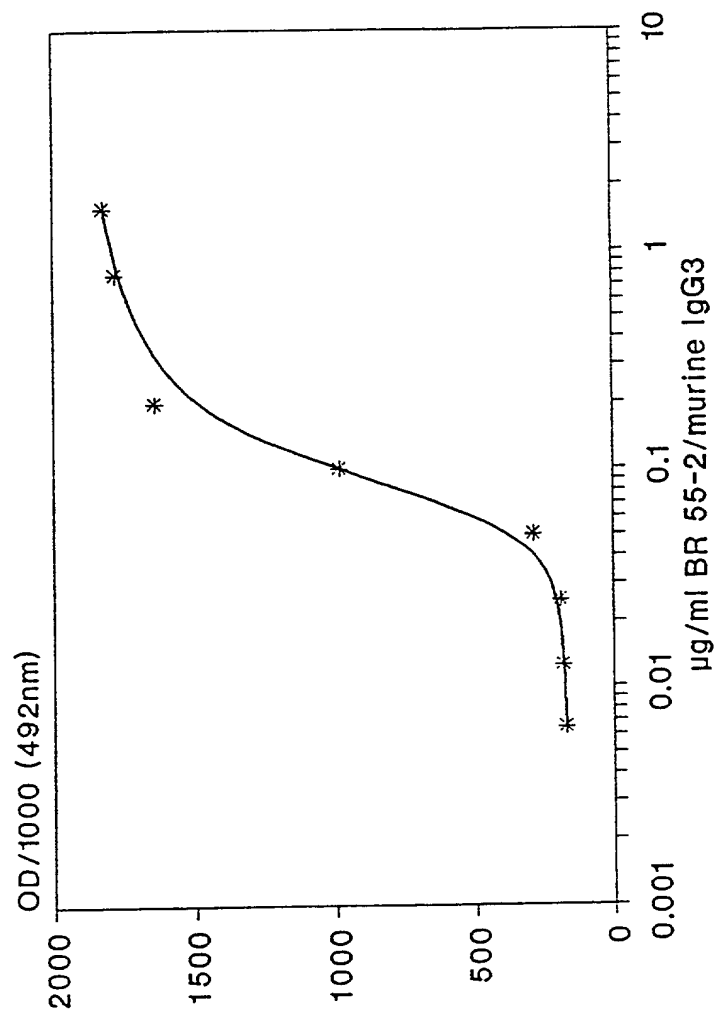


Figure 28
ELISA for the determination of
BR 55-2/chimeric human IgG1
in human serum - standard curve

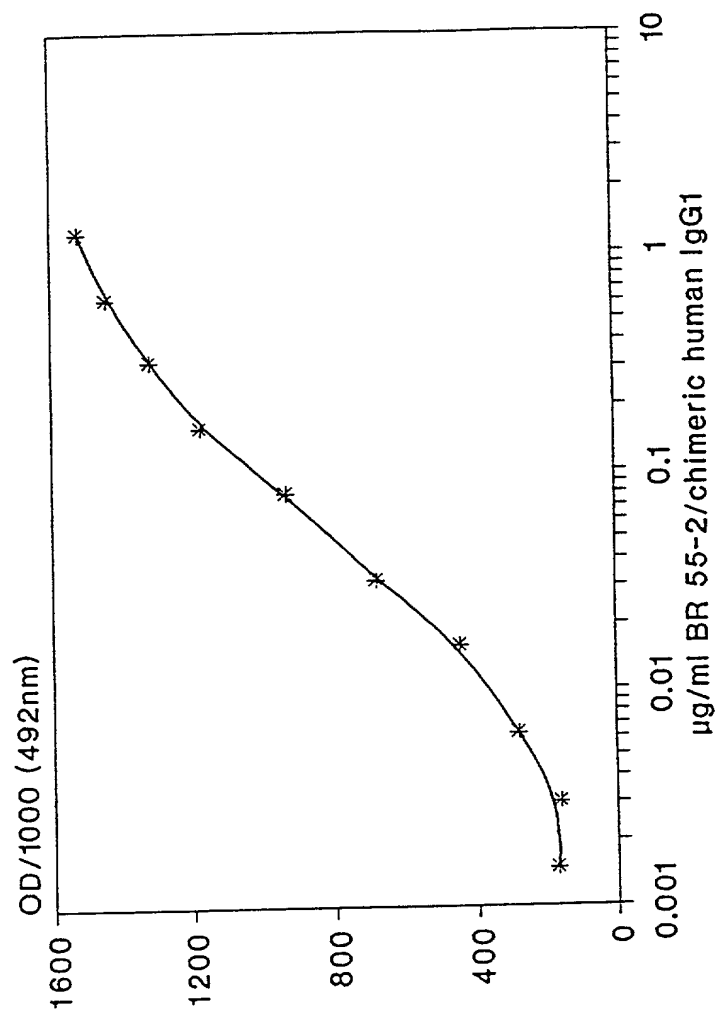


Figure 29
ELISA for the determination of
BR 55-2/chimeric human IgG3
in human serum - standard curve

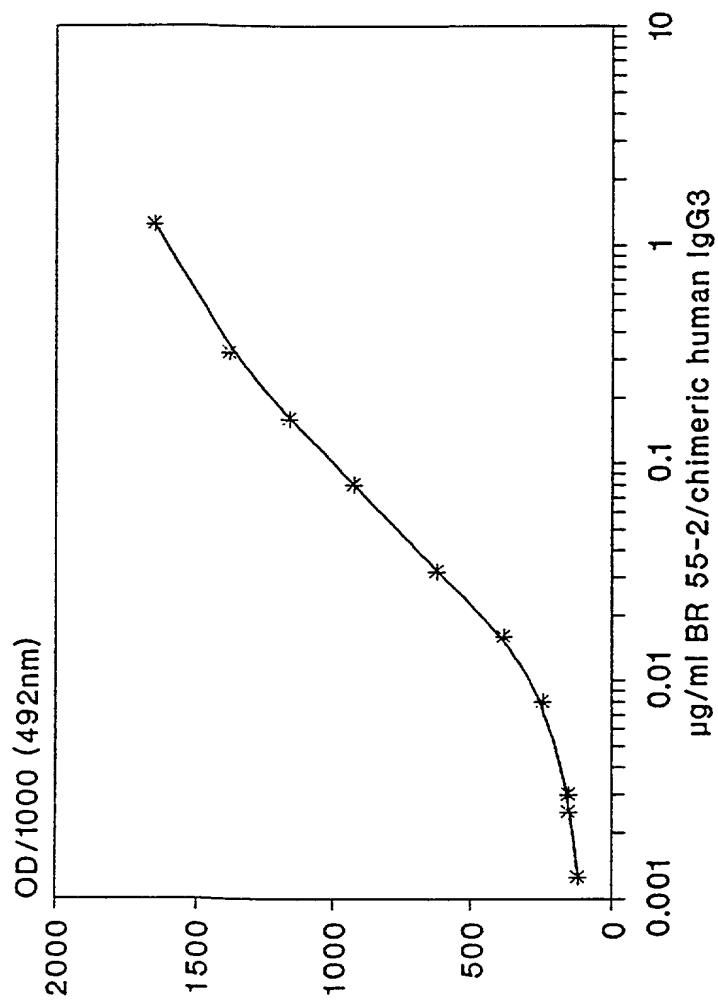


Figure 30

Binding of BR55-2/chimeric IgG1 and
BR55-2/humanized IgG1 to anti-id BR55-2#E4

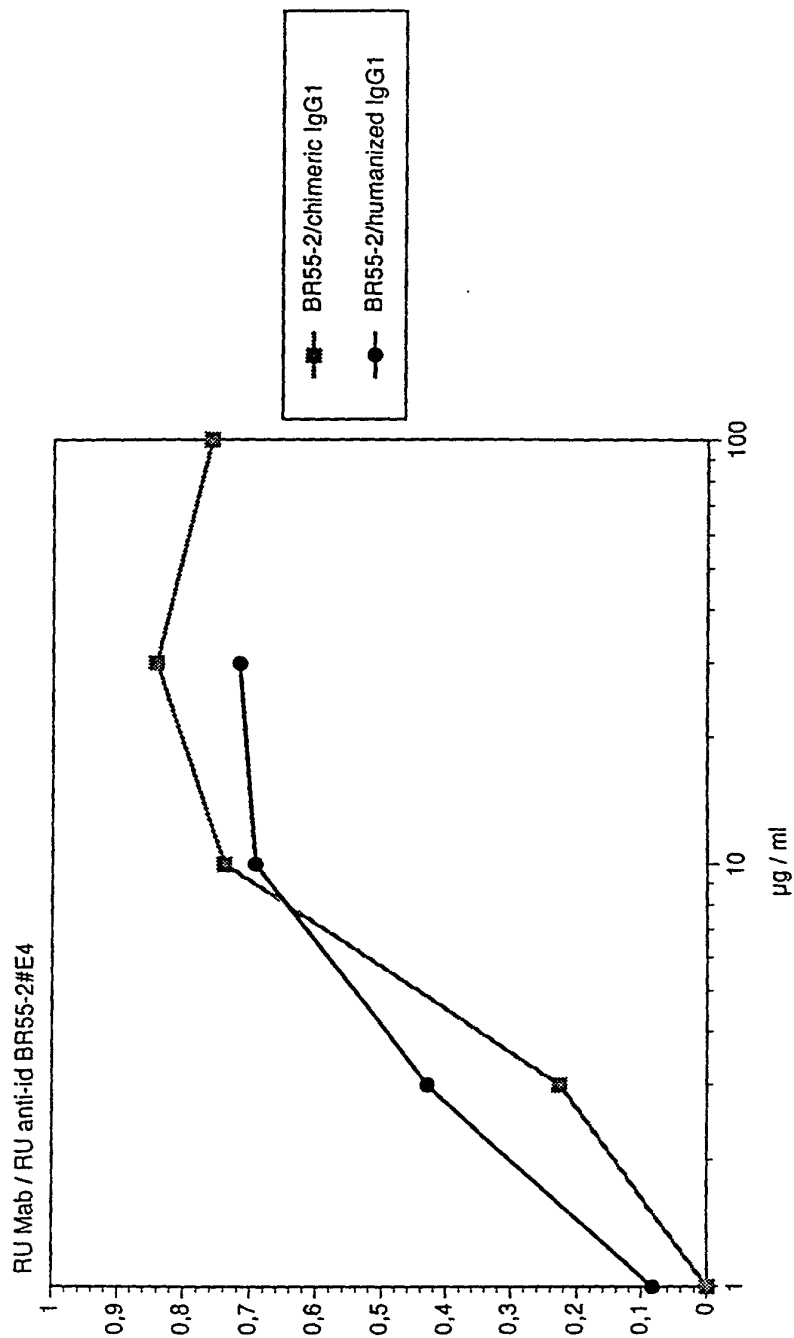


Figure 31
Binding of BR55-2/chimeric IgG1 and
BR55-2/humanized IgG1 to anti-id BR55-2#C11

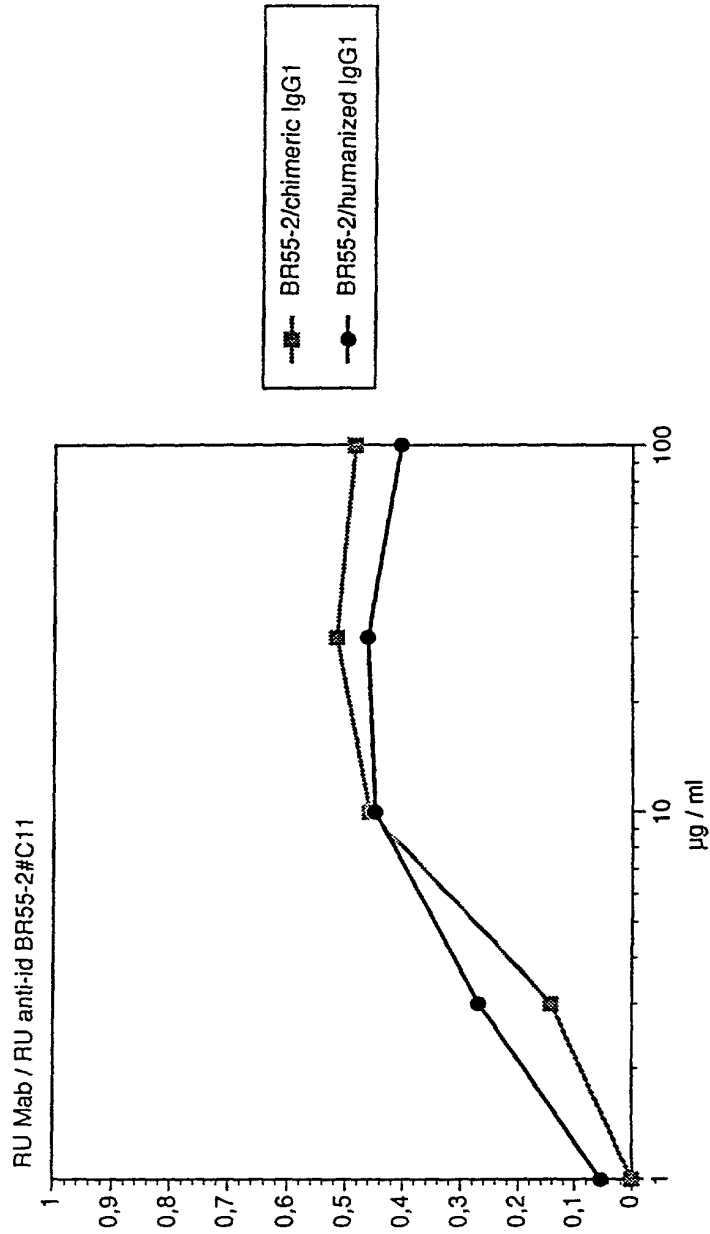


Figure 32
Binding of BR55-2/chimeric IgG1 and
BR55-2/humanized IgG1 to anti-id BR55-2#B3

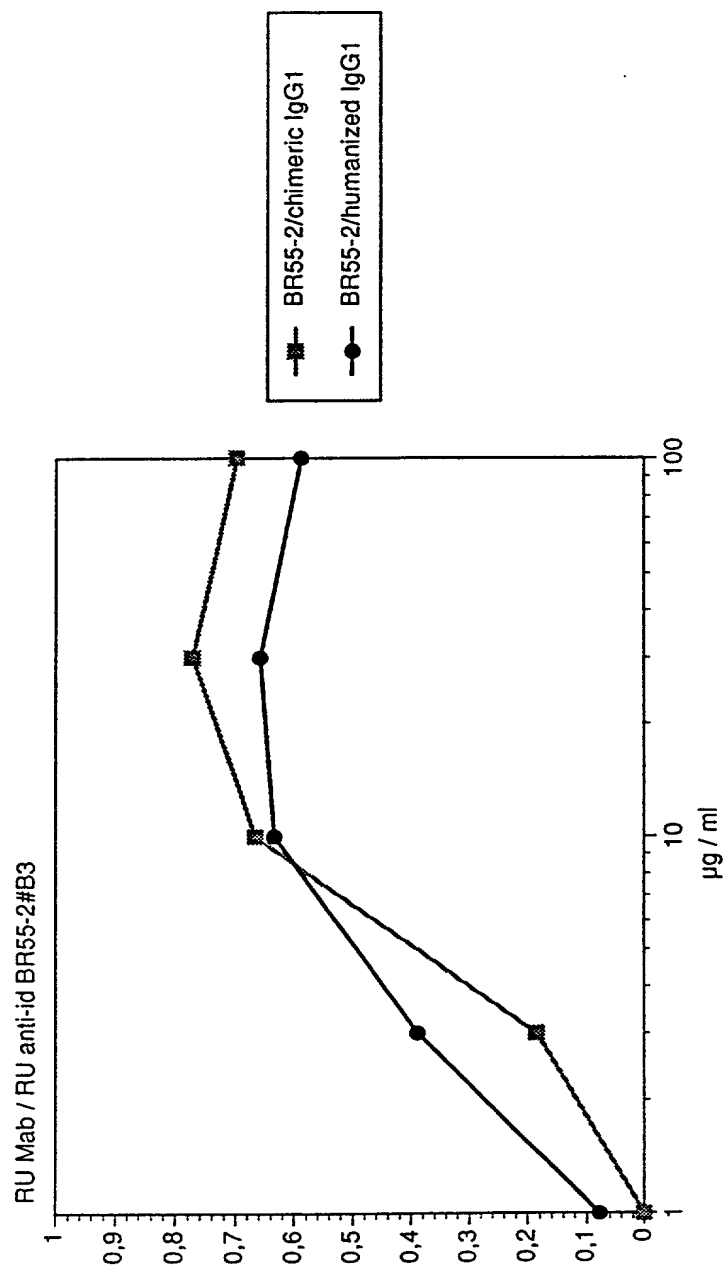


Figure 33
Binding of BR55-2/chimeric IgG1 and
BR55-2/humanized IgG1 to anti-id BR55-2#B9

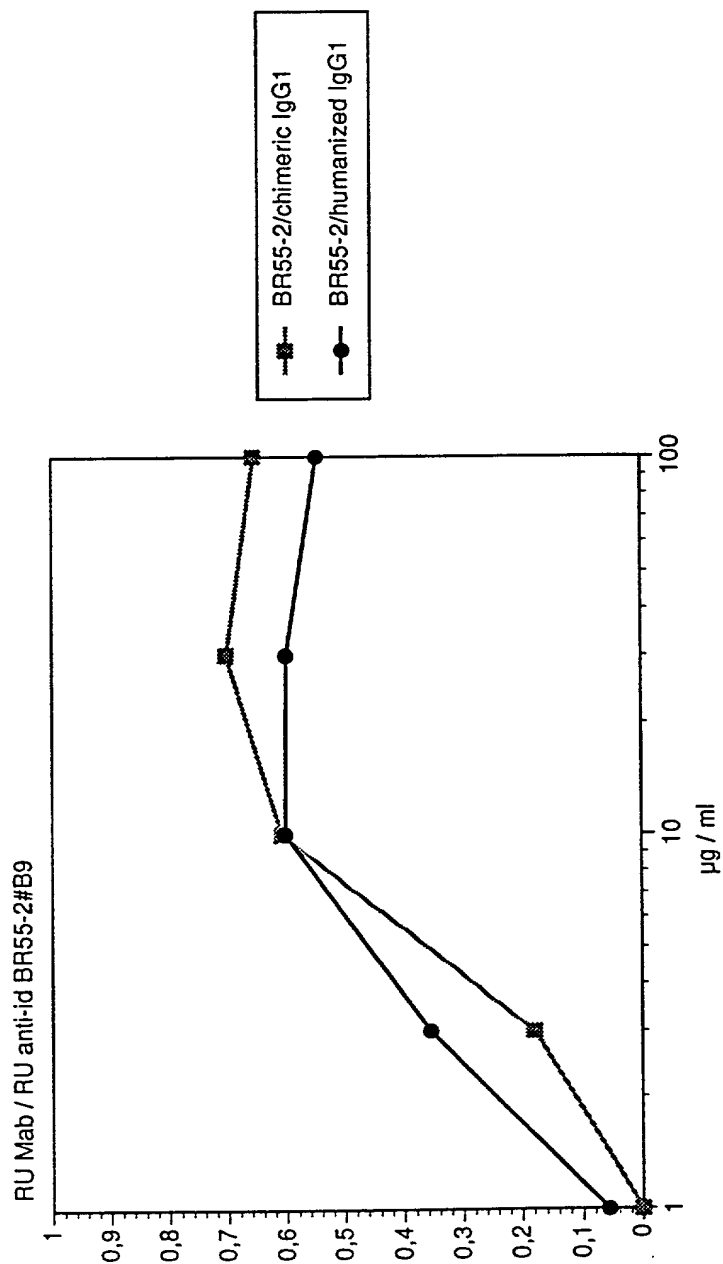


Figure 34
Binding of BR55-2/chimeric IgG1 and
BR55-2/humanized IgG1 to anti-id BR55-2#G6

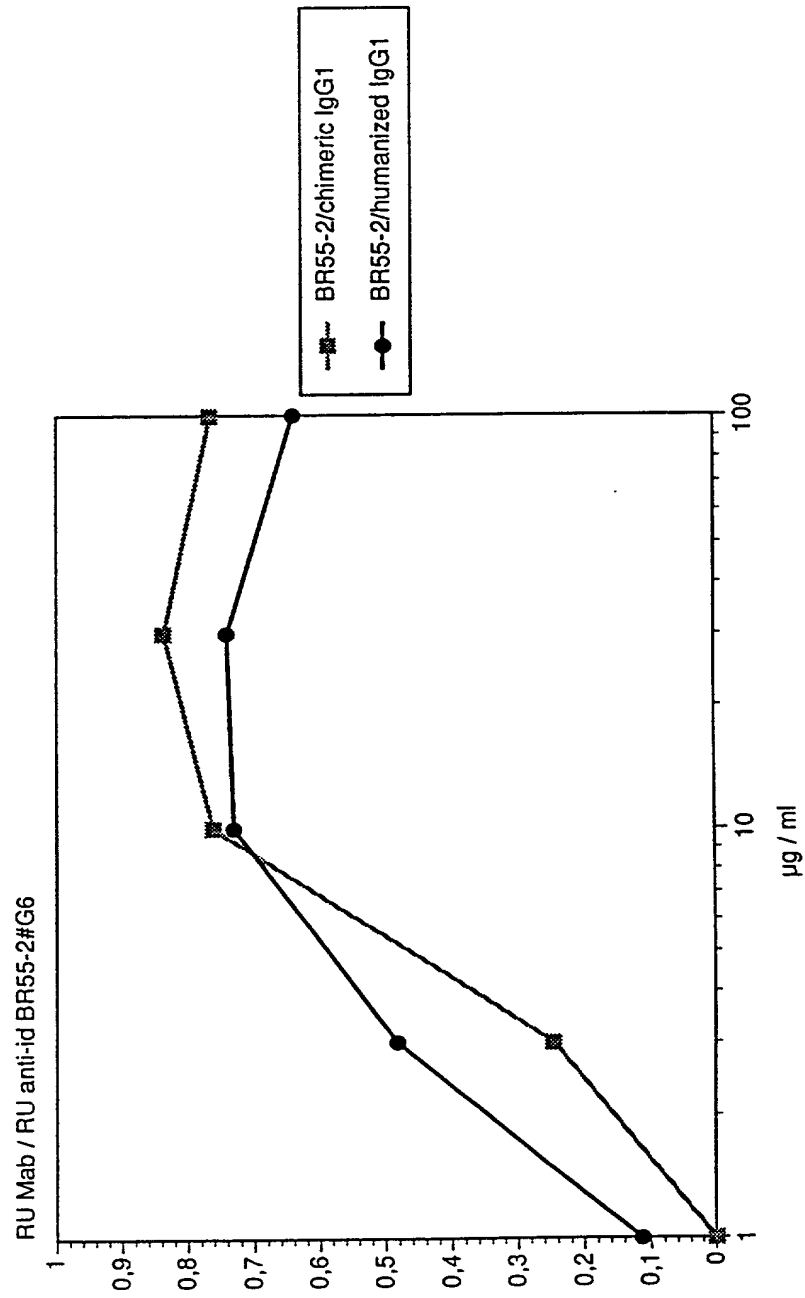


Figure 35
Binding of BR55-2/chimeric IgG1 and
BR55-2/humanized IgG1 to anti-id BR55-2#G9

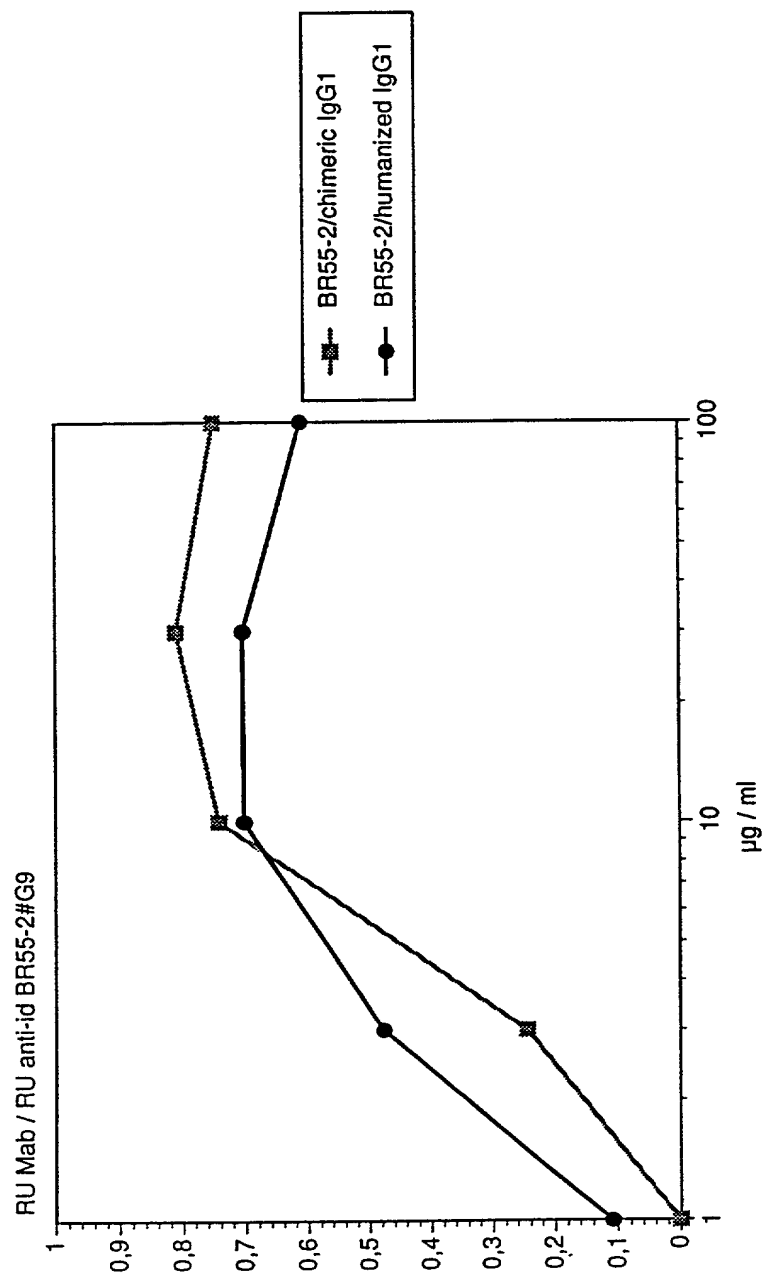


Figure 36
ELISA for the determination of
BR 55-2/humanized IgG1 in human serum
Standard curve

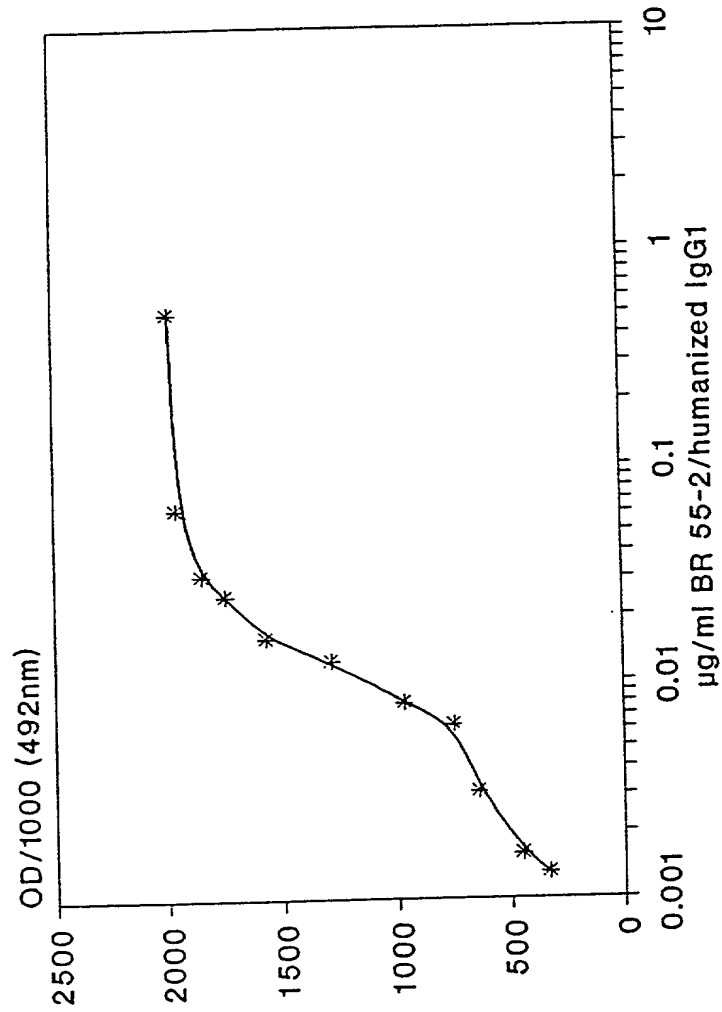


Figure 37
Binding competition of BR55-2/murineIgG3
to a-ID BR55-2 by BR55-2/chim.human IgG1
and BR55-2/chim.human IgG3

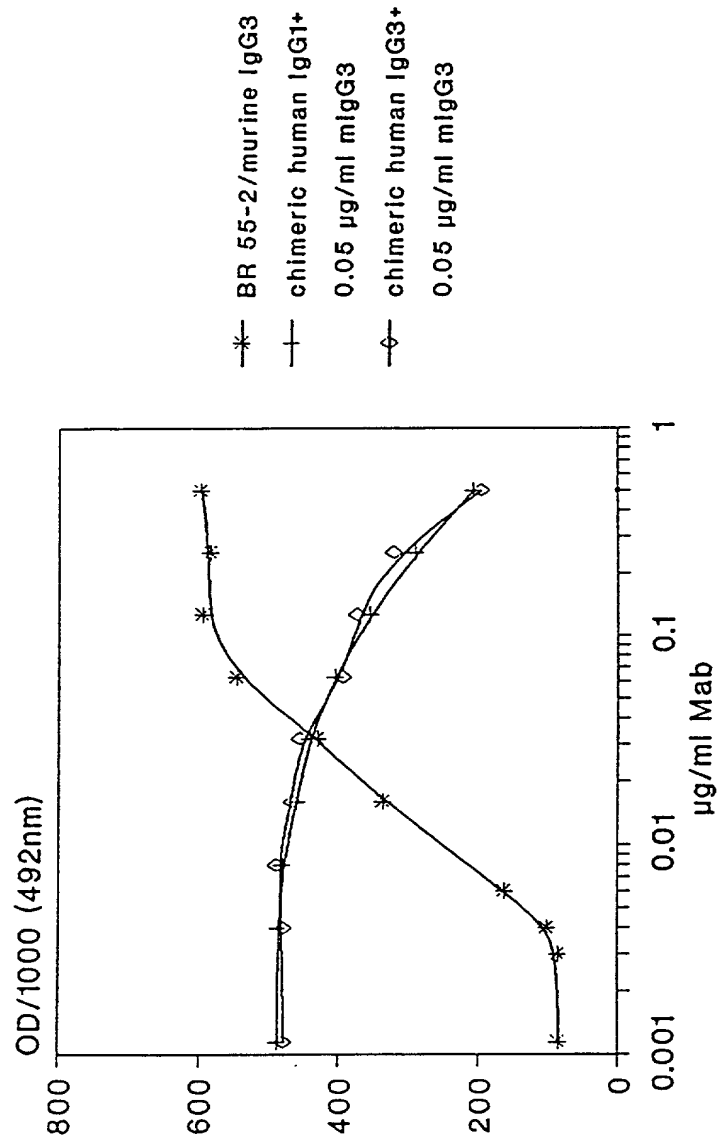


Figure 38
Complement dependent cytotoxicity
mediated by BR 55-2/murine IgG3
Inhibition by anti-ID BR 55-2 E4

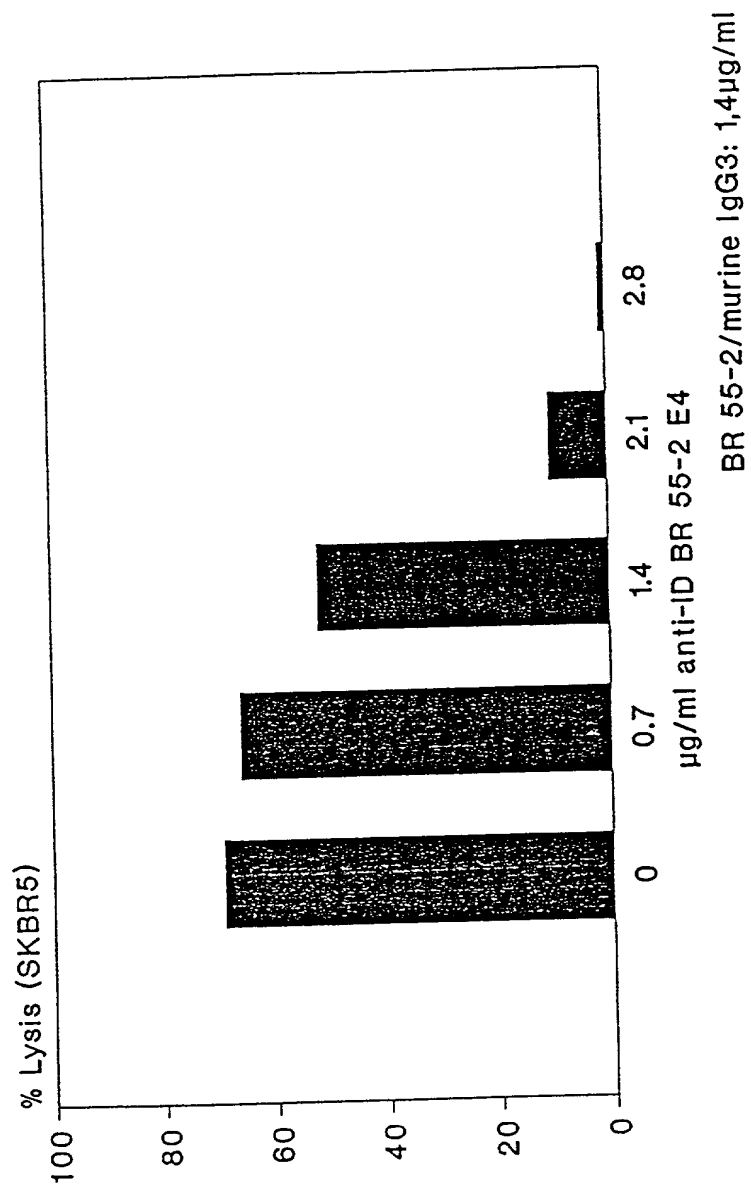
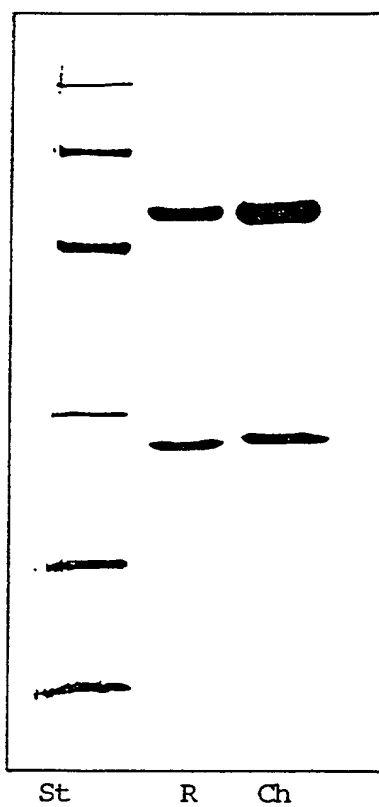


Figure 39

S D S - P A G E
Reducing conditions



St = Standards

R = Reference Mab

Ch = BR55-2/chimeric human IgG1